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FLORA OF CORN MEAL

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INTRODUCTION

Corn meal as it comes from the mill carries the mycelia of certain fungi which infect unground grain. In addition, numerous species of molds and bacteria, present in spore form as contaminations upon the surfaces of sound kernels or as saprophytes in partially spoiled grains, are recoverable by routine cultural examination of the finished meal. Many experiments, extending over several years and including the work of various members of the Microbiological Laboratory, show that certain groups of organisms are practically always abundant in such cultures. Other species are usually present, but in smaller numbers, and many forms are obtained occasionally as accidental contaminations. In undertaking to study this complex flora, it may be possible to determine by routine culture the species represented and something of their relative abundance in the sample, but the list so obtained gives little information as to the relative importance of the individual species as causes of spoilage in the product.

The culture media commonly used in such routine examination of foodstuffs present conditions for the growth of microorganisms which differ greatly from those found in corn meal. The nutrients used in preparing such media are selected because they are readily assimilable to most organisms. These nutrients appear in solution or in jelly-like masses which contain high percentages of moisture. Corn meal, on the other hand, presents a range of composition, according to Winton and his associates (*8*),[1] approximately as follows: Moisture, 10 to 18 per cent, but under usual commercial practices ranging from 12 to 15 per cent; protein, 5 to 10 per cent; fat, 1 to 5 per cent, according to the method of milling; nitrogen-free extract, including starch and sugar, 68 to 78 per cent. Of the nitrogen-free extract, sugars constitute perhaps 3 per cent, and gums and dextrin, some of which are readily fermentable, perhaps an equal quantity. In dealing with this product as a substratum for organisms, the percentage of water found is an important limiting

[1] Reference is made by number (italic) to "Literature cited," p. 188.

factor. Obviously this product, even at its maximum moisture content, presents a marked contrast to laboratory media as usually prepared. Nevertheless, corn meal has been so often found an unstable product that it is commonly milled only for consumption within a few weeks or by methods intended to eliminate the most readily fermentable portions of the grain.

Under ordinary conditions of handling, spoilage in this product appears in one of the following forms: Souring, rancidity, mustiness, the formation of clumps or balls, extensive concretions which may involve the solidification of an entire bag, or the formation of a hard, cylindrical outer mass with the center loose and mealy. Heating occurs only in the wettest samples. Much corn meal, if held beyond a very short period, develops a musty, moldy, or sour odor and shows occasional balls or masses of meal held together by mold, which bring about losses in palatability and market quality in the product. Such changes as rancidity and the formation of extensive concretions into moldy masses are so obviously due to high moisture content and involve such losses that they have been almost eliminated from commercial practice. When losses occur the meal is found to carry more than a critical moisture percentage. This may be due either to milling corn which is insufficiently dried or to the storage of the meal under conditions which will maintain a moisture content above the danger point. For the samples used in all series reported here this figure was approximately 13 per cent (*2*).

CULTURAL EXAMINATION

In routine cultural examination reported here, plain agar was used for bacterial counts, wort agar for mold counts, and dextrose-litmus shake agar to determine acid, gas, and anærobic growth. The presence of particular organisms was determined by the use of special methods on special media. Experimentation covered a range wide enough to justify the restriction of routine cultures to the media already noted.

After comparative study of many series of cultures, Table I is introduced as giving a group of cultural results fairly typical for commercial meal in sound, merchantable condition. The nine samples reported were purchased in different retail stores of Washington, D. C., during October and November, 1920. Four of them were yellow and fairly coarsely ground. The white meals were softer or more finely ground. All were bolted. All showed by microscopic examination traces of both bran and germ, although these portions were scanty in certain samples. The history of the samples was not obtained.

These samples were sound in appearance and odor. There was no evidence of the multiplication of microorganisms. Among the bacterial colonies micrococci, members of the mesentericus and of the colon-aerogenes groups were characteristically present. Special tests in cab-

bage juice showed, in four of the nine samples, the presence of lactobacilli with the morphology and cultural characters of the organism of pickle and sauerkraut fermentation. No bacterial colonies were obtained in plain agar from two of the samples. A duplicate of sample 9 proved equally negative. Mold colonies were obtained in all samples. These represented in varying proportions *Aspergillus repens* De Bary, *A. niger* Van Tieghem, *A. flavus* Link, Fusarium, various mucors, and unidentified colonies.

TABLE I.—*Results of cultural examination of commercial corn meals*

Sample No.	Bacteria per gram on plain agar.	Molds per gram on wort agar.	Bacteria per gram on dextrose-litmus agar.	Acid colonies.
				Per cent.
1	10, 000	10, 000	16, 000	50
2	5, 000	1, 000	.....	.....
3	55, 000	12, 000	42, 000	60
4	60, 000	10, 000	13, 000	50
5	70, 000	400, 000	10, 000	50
6	5, 000	20, 000	8, 000	60
7	.....	3, 000	.....	.....
8	10, 000	11, 000	4, 000	100
9	.....	5, 000	3, 000	30

A more extensive series of studies was conducted in cooperation with the Plant Chemical Laboratory of this bureau. The general results of this experiment are described elsewhere (*2*). In brief, during the spring of 1920, a series of bags of meal were prepared for this storage experiment from corn bought by the mill in the regular course of business. This grain, while sold as No. 2, was obviously wet and barely passable as a fair product. Infected and even badly decomposed ears were not uncommon among the ears of corn received in bulk. Although the lots of meal included were milled at water contents varying from 12.7 to 16.18 per cent, the conditions of storage were such that no spoilage determinable by the senses took place. Cultures were made from the meal as freshly ground in April, then, beginning May 5, once each week until July. In all these cultures no evidence of multiplication of either mold or bacteria was found. It was, therefore, possible to follow the relative numbers of viable organisms in the various groups from the time of grinding through the four months of storage.

In the freshly milled samples the average count of colonies of bacteria upon plain agar was about 1,000,000 per gram of meal, with variations from 600,000 to 1,600,000. Upon wort agar the count of mold colonies averaged about 100,000 per gram of meal, with variations in different samples from 70,000 to 160,000. Of the bacterial colonies observed about 60 per cent were acid producers.

For comparison a special series of samples were prepared by adding 5 per cent of meal made from corn markedly rotted with Diplodia and Fusarium. In the freshly ground meal of this series the bacterial count upon plain agar was about 2,600,000. The count of mold colonies upon wort agar was about 110,000. About 70 per cent of the bacterial colonies were acid producers.

After storage for approximately one month (May 20 and 21) samples from a particular lot of five bags of the regular meal showed an average count of 108,000 bacterial colonies and 15,000 molds. Samples from the same bags on June 30 showed an average count of 12,600 bacterial colonies, and 7,600 mold colonies. Without placing emphasis upon exact figures, these cultural results are fairly typical of the mass of figures obtained from cultures made weekly from representative samples involving the whole series of 88 bags of meal. These figures are readily comparable with those obtained from commercial samples (Table I). Discrepancies which occur may perhaps be accounted for by the fact that samples 3, 4, and 5 were evidently the product of local mills, sold fairly quickly after milling, while samples 2, 7, and 9 were clearly the product of special processes and handled under conditions involving much slower distribution.

In this lot of meal, therefore, the conspicuous change due to storage was the drop in the number of viable organisms to about 1 per cent of the original number of bacteria and perhaps 10 per cent of the original number of molds. The larger part of this decrease occurred during the first six weeks, with a slow reduction throughout the succeeding periods.

In connection with the study of these figures, data obtained by Thom and Stiles (unpublished) in examining Winton's (*8*) samples[1] in 1914 were restudied and compared with the results here considered. Winton's corn meal varied in initial moisture content from 19.27 to 10.79 per cent. In those lots of meal (A, B, and C) carrying moisture markedly above 13 per cent, the evidence of multiplication of molds and bacteria was clearly discernible. Musty odors and balls of meal held together by mold were present in every sample. In cultures, the count of colonies of molds and bacteria reached 13 million in the wettest lot. Of these several million were *Aspergillus flavus*. The predominant organisms were molds rather than bacteria, but there was fairly clear evidence of some bacterial multiplication at the higher water percentages.

In the roller-ground samples of lots D, E, F, which did not spoil and whose water percentage was near to or less than 13, the total counts found by Stiles approached very nearly those already given in this paper. These examinations began too late in the storage period to show that part of the bacterial flora which dies off rapidly. The stored samples still showed some acid organisms, but micrococci and aerobic spore

[1] Samples of the meal studied were examined bacteriologically by G. W. Stiles, formerly of the Bureau of Chemistry, and for mold activity by Charles Thom, then in the Bureau of Animal Industry (*8, p. 25*).

formers of the mesentericus group formed the majority of the bacteria obtained.

In the lots with moisture content decreasing toward 13 per cent there was progressive reduction in the number of active species of molds. Extensive experimentation showed clearly that *Aspergillus repens* was the agent which formed the balls of meal loosely held together with mold hyphae, which characterized meal containing barely enough water to start spoilage. In another series of experiments *A. flavus* began to be active only in samples containing about 16 per cent of water. Yeasts, mucors, and Penicillia were reported by Stiles only in the sample carrying about 19 per cent of water.

During the examination of the preliminary samples in the 1920 experiment, an effort was made to identify the groups or actual species represented. As a matter of routine, inoculations were made from each flask prepared for diluting plates (consisting of 5 gm. of the meal to 45 cc. of sterile water) into the following media: Plain milk, gelatin, and litmus lactose broth. Smears were also made on Endo's agar in each instance. In every case there was prompt coagulation of the milk, with extrusion of whey, but no digestion of curd. Pink rings formed near the surface. Gelatin was liquefied in every instance, and acid and gas formed in all broth tubes. Growth in Endo's media indicated the presence of *Bacterium aerogenes* Escherich. Further cultural studies showed that *Bact. aerogenes* was the predominant bacterial species present in all these samples. This predominance was maintained throughout the series of examinations made. Microscopical examinations of smears made in each case, however, showed the presence of spore-bearing bacteria, especially the mesentericus group, and micrococci of various kinds. Dextrose agar tubes often contained colonies growing deep in the media, indicating the presence of anaerobic bacteria. Yeasts were found in all samples, their growth being largely of the mycoderma type. The plates showed many mold colonies. Various mucors, species of Fusarium, *Aspergillus flavus*, *A. niger*, and occasional green Penicillia were observed. The species of molds present on the plates varied from period to period and with the sample. Molds were always more numerous on plates made from meal to which Fusarium and Diplodia had been added, but growth on these plates did not show dominance of these particular forms.

Evidence of the effect of bolting upon the abundance of organisms was furnished in the 1920 experiment by the examination of samples of two series of five bags each, representing a single lot of meal, one-half of which was bolted and the other half unbolted. The bolting to which these samples were subjected removed a considerable part of the bran but little of the germ from the meal. After one month of storage, the bolted meal showed an average of 34,000 bacterial colonies and 20,000 mold colonies. The unbolted samples showed 108,000 bacterial colonies

and 15,000 molds. This observation was confirmed by a restudy of Stiles's unpublished examination of Winton's (*8*) samples. Of every lot of corn handled, part was ground in a stone mill without sifting or bolting and part was carefully "degerminated" and "roller" ground. In the bolting process all of the bran was taken out, and many of the samples consisted almost completely of horny endosperm. In that part of this series made up of meals in which no multiplication of microorganisms occurred, bolting consistently reduced the cultural count of microorganisms below that of the stone-ground meal. Frequently the number found in the bolted meal was less than one-tenth of that in the stone-ground meal.

By removing the bran, bolting takes away the largest area of contamination with saprophytic organisms. The tip of the kernel and the germinal area carry the majority of the infections found in corn. Study of many samples of corn over a period of years shows that invasion of the germinal area by molds is not uncommon in corn which has not been fully matured or has not been promptly and thoroughly dried. Samples have frequently shown the invasion of the germ in every kernel by *Aspergillus repens*. Recently samples representing a bulk shipment have shown nearly every grain to contain one or the other of two species of Penicillium. Meal therefore may be so milled and sifted or bolted as to remove the larger part of all contaminations, as well as those mold infections which do not involve general disintegration. The cleaning process before milling removes the grains thoroughly rotted by Fusarium and Diplodia. Corn has still been seen going into the rolls of a mill in which the low grade of the stock could not have been concealed if it had passed through a stone mill without being bolted. The product, however, was going into human food without showing tangible evidence of the low quality indicated by the unground grain. In other words, the fractional milling of low-grade grain makes possible such separation as turns the infected portions of the grain into oil stock or cattle feed and the solid or horny portions which are less obviously damaged into meal.

The literature of maize deterioration is reviewed by Alsberg and Black up to 1913 (*1*). The activity of Fusarium and Diplodia as causes of rotting in ear corn was discussed by Burrill and Barrett (*3*) and that of Diplodia alone by Heald, Wilcox, and Pool (*4*).

More recently McHargue (*6*) has studied the activities of certain fungi and their relation to commercial conditions in the handling of the product. Excessive moisture in the grain is regarded as the limiting factor in most cases of such spoilage. The factor of temperature must not be overlooked. The moisture content limit may be materially increased during the winter without evidence of the activity of microorganisms. The agents of spoilage in all the cases under review were primarily molds. The results already given in this paper harmonize in general with those

of McHargue. It has been possible, however, to go farther and indicate more clearly the groups of organisms regularly present and to record the conditions under which certain of them become active factors in spoilage.

Routine mass or dilution cultures show that certain molds are recoverable from practically all samples of meal. Among these are *Rhizopus nigricans* Ehrenberg and some of the mucors which frequently overgrow plate cultures within two days of incubation, although they probably are present only in spore form in the meal. Syncephalastrum, belonging to the same group, is not uncommon. *Aspergillus flavus* and *A. niger* are only occasionally visible factors in the infection of the unground grains, but they always appear as rapidly growing colonies in the mass or dilution cultures made. The brown masses of *A. tamari* Kita are commonly found with *A. flavus*. *A. fumigatus* Fres. and *A. terreus* Thom are frequently present but are quickly overgrown by the more active species already mentioned. *A. repens*, though practically always present, can be found only by careful search in the presence of these rapidly growing forms.

Several strains of Penicillium are found in meal cultures. Penicillia of the group with submerged orange mycelia and of the Citromyces group are probably most common. *Penicillium expansum* Link is reported by McHargue. *P. oxalicum* Thom and Currie is found in many samples of meal, but rarely in miscellaneous cultural work. Strains related to *P. luteum* Zukal and *P. purpurogenum* O. Stoll are frequently present but usually indicate soil contamination rather than active growth in the corn or meal. One sample of corn rotted by a member of this series has been examined, but the conditions shown clearly indicated that the product had contained high percentages of moisture at the time the rotting occurred.

Colonies of Fusarium develop from almost every sample of meal. Infections of this group are so abundant that conidia or grains of meal containing living hyphae are rarely absent. Cladosporium and Alternaria are frequently found but represent spore contamination rather than infection. The other organisms observed in culture from time to time appear to represent excessive contaminations with spores due to unfavorable conditions in the handling of the product, or, in certain species, to actual infection of the grain locally by the mold.

The bacteria found in the fresh samples here considered were predominantly *Bacterium aerogenes*. Certain other organisms have been regularly obtained in culture. When the necessary moisture is present, souring is so characteristic of the product that Round and Gore (7) found the addition of 3 per cent of fresh meal an adequate starter to insure the dominance of lactic acid fermentation in potato silage. Lacto-bacilli were present in four of the nine lots reported in Table I. According to unpublished records in the Microbiological Laboratory, Round found organisms of this group abundant also in fresh meal, but occasionally

absent in old meal or meal made from old and thoroughly dried corn. Micrococci are constantly encountered in culture but have not been typed. Aerobic spore formers of the mesentericus group are always present, and in spore form they constitute the larger part of the living bacteria in some meals after long storage.

This was clearly demonstrated by a series of experiments upon the possibility of producing a sterile meal with steam, dry heat, or both (unpublished cultural results of Ruth B. Edmondson). The spores of this group survived more heating than could be applied under practical working conditions to the product. Aside, however, from meal so wet as to be unmarketable, these experiments show no evidence of bacterial activity. One sample of apparently sound yellow meal showed the presence of *Bacillus niger* Migula in such extensive numbers that masses of meal placed upon culture media were promptly overgrown and with the agar turned bluish black with this species. The meal was contributed by Dr. S. S. Adams, of Washington, D. C., who reported the feces of a child apparently well to have been blue when fed this meal.

When, however, corn or meal is bottled and incubated at laboratory temperature (20° to 30° C.), those species capable of developing under the conditions presented show active growth. In the authors' series such growth was not detected by physical appearance in products carrying less than 13 per cent of moisture. Certain stone-ground samples of Winton's series (*8*) showed some evidence of mold activity below that figure. Measurable changes in quality certainly occur in such meals during storage. Some experimental results have suggested the possibility that these changes in such meal are due to the distribution of infected material throughout the mass by the grinding of infected corn. This conflicts with the current trade belief that the natural enzyms of the germinal area are the chief causes of such deterioration, but reflects the findings of Hoffer (*5*) and his coworkers that even selected seed corn may be extensively infected. Examinations of commercial samples in the Microbiological Laboratory have shown extensive development of molds within the grain itself in corn of other than the higher grades.

In samples carrying 14 to 15 per cent of water the formation of balls and concretions in the meal begins to be evident. The principal agent in their formation appears to be *Aspergillus repens*, although many difficulties are encountered in fixing a minimum moisture percentage for the activity of this species. Changes involving the development of mold mycelium in the meal begin within the limit of 13 to 15 per cent of moisture. Incubation at 20° to 30° C. merely accelerates changes which would progress more slowly in colder places. Moist chamber experiments with meal inside this range of water content show the presence of active mycelia of more than a single species, but principally *Aspergillus repens*. When the percentage of moisture reaches 16, several species are clearly able to grow. Special studies with *Aspergillus*

flavus show that very little development of this species occurs below 16 per cent, but that from 16 per cent upward development of this species rapidly increases and the number of forms capable of growing rapidly rises. Among the characteristic saprophytic molds observed under these conditions, in about the order of their abundance under the conditions, are *Aspergillus repens*, *Aspergillus flavus*, *Actinomyces* sp., *Penicillium* sp. and *Citromyces* sp., *Fusarium* sp., *Aspergillus candidus*, *Aspergillus ochraceous* Wilhelm, *Aspergillus tamari*, and *Aspergillus niger*.

Bacterial activity appears to be a concomitant of the disintegration due to mold action in such rotting processes as this. As indicated by Bailey and Thom (*2*, *Table I*), active disintegration by molds is accompanied by an increase in the water percentage of the sample. Bacteria follow rather than initiate the process in the samples studied, thus becoming a small factor in the merchantable product.

Throughout this investigation a close correspondence has been observed between the flora of deterioration in unground corn and the flora of the milled product.

SUMMARY

In seeking possible causes for the well-recognized instability of corn meal, cultures show considerable numbers of molds and bacteria to be generally present. Among these the following species of molds were characteristic of many series of cultures: *Fusarium* sp., *Aspergillus repens*, *A. flavus*, *A. tamari*, *A. niger*, *Citromyces* (or *Penicillium* section *Citromyces*) sp., *Penicillium oxalicum*, *P. luteum* varieties, *Mucor* sp., *Rhizopus nigricans*, and *Syncephalastrum* sp., together with various yeasts and yeast-like fungi. Among bacterial groups, the colon-aerogenes group and lacto-bacilli were most abundant in fresh meal. Aerobic spore formers and micrococci were always present and persisted in the stored product.

Within the range of composition found in merchantable meals, no bacterial activity was detected. Only one grade of unbolted meal showed signs of mold development below 13 per cent of moisture. Above 13 per cent moisture, *Aspergillus repens* begins to be an active agent of spoilage somewhere between 13 and 15 per cent of moisture, varying with the form of milling practiced. Several other species of molds are active in meal containing 16 per cent moisture; and numerous forms, including some bacteria, develop when 18 to 20 per cent of moisture is found.

Many samples of corn are found to carry extensive infections with Fusarium, Diplodia, *Aspergillus repens*, or Penicillium, especially in the germinal area and in the tip of the kernel. These sections of the kernel are removed in varying degrees by different milling systems. The bolted meals examined show a corresponding reduction in count of viable organisms as shown by culture.

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HOPKINS HOST-SELECTION PRINCIPLE AS RELATED TO CERTAIN CERAMBYCID BEETLES

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INTRODUCTION

In connection with the reported dying of lodgepole pine (*Pinus contorta* Loud.) over extensive areas in northeastern Oregon caused by the mountain pine beetle (*Dendroctonus monticolae* Hopk.) and the threatened invasion by this beetle of the adjacent areas of yellow pine (*Pinus ponderosa* Laws.), detailed investigations were made by the Bureau of Entomology under the direction of Dr. A. D. Hopkins. Manuscript reports of these investigations, submitted in the summer of 1910, showed that the infestation by the beetle in the lodgepole pine was so extensive that there was no hope of controlling it, but that the comparatively small amount of infestation in the valuable stands of yellow pine was such as to warrant the undertaking of control, provided the beetle did not migrate from the lodgepole pine to the yellow pine.

In a letter from Dr. A. D. Hopkins under date of July 30, 1910, relating to a manuscript report of Mr. H. E. Burke, the following statement occurs which appears to be the first written reference to the host-selection principle:

> The more I consider the various features of the problem, the more I am convinced that it is entirely practicable to protect the yellow pine, even if we leave all but the immediately adjacent lodgepole pine to take care of itself. This is based on my belief that the majority of the broods of the beetles which have been breeding in the lodgepole will continue to confine their attack to that species, and gradually diminish with the reduced supply and their increased struggle to adapt themselves to the yellow pine. I may be wrong in this, but it is a matter worthy of careful consideration. Remember, that in all these years, there has been no marked or general migration of beetles from lodgepole to the yellow pine. Therefore, it appears that the broods which are most dangerous to the yellow pine are those which have been breeding in it, and that these are the broods we will have to deal with mainly in our efforts to protect the best bodies of yellow pine.

The control operations that were carried on during the following year, 1911, were confined mainly to the yellow pine area. In manuscript reports by Messrs. W. D. Edmonston and George Hofer on a special examination of the yellow pine and lodgepole pine areas in the summer and fall of 1913, it is stated by Edmonston:

> In 1912 the examination of the areas on which insect control work was carried on during April, May, and June, 1911, showed an average reduction of the infestation on the entire area, 76,430 acres, of close to 85 per cent.

Examinations made this season, 1913, show a still greater reduction in the infestation; in fact, the infestation is so light that it is actually less on the treated areas than it is throughout any other area on this Forest.

and—

There was no reoccupation of the treated areas by broods from the lodgepole infested trees at higher elevations.

and by Hofer—

As we reached the summit near the North Powder Peaks we attained an altitude of 8,000 feet; the elevation at the Sheep Ranch is about 4,000 feet. From the summit of this divide for a distance of 10 miles north, 10 miles east, and 16 miles west we noted heavy infestation, both old deadings and the new work also, in both the lodgepole pine and white bark pine, especially on both slopes of Antone Creek.

No new infestation was found on the treated areas on Anthony Creek, Camp area.

This seemed to furnish substantial evidence that the principle would hold.

The principle as defined by Dr. Hopkins [1] is that an insect—

species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted.

In order to secure further evidence relating to this principle, the writer, after consulting with Dr. Hopkins, began a series of experiments in 1914 with insects which infest two or more species of wood. The wood-boring Cerambycidae, or long-horned beetles, offered material which was very well adapted to the conduct of such experiments. Many species were easily available which exhibit great diversity in their selection of hosts in nature, as illustrated by those breeding exclusively in a single species of plant and those apparently attacking almost any wood. This variation in host habits at once brought up the following questions: Will those species confined to a single host live in any other, and do the individuals coming from a certain plant of those species breeding in a variety of hosts select the same species of plant on which to oviposit? Again, if such is the case, how do these host strains originate in nature?

As these experiments progressed new problems came up demanding a broadening of the experiments from year to year until, during the season of 1918, over 100 individual experiments were in progress. Fourteen species of insects and 21 species of plants were used, combining to form 45 host strains. It was thought desirable to conduct experiments on more species rather than more intensive experiments on a few species. It will be noted that certain experiments were not carried as far as others, due to the fact that time was not available or due to the absence of the writer at the critical time. At present several points remain to be conclusively settled, and investigation of these will be continued another year or so. Nevertheless it is believed that sufficient data have been accumulated to show definitely the extent to which the influence of the host applies to these insects.

[1] HOPKINS, A. D. ECONOMIC INVESTIGATIONS OF THE SCOLYTID BARK AND TIMBER BEETLES OF NORTH AMERICA. *In* U. S. Dept. Agr. Program of Work, 1917, p. 353. 1916.

HISTORICAL

Very few references to the adaptation of insects to their host plants or the variation in their selection of host plants can be found. The most important paper dealing with the subject is that by Pictet.[1] This author shows by several examples, (*Ocneria*) *Porthetria dispar* for one, that caterpillars of the second and third generations may be made to change their preferred food plants and that the adults reared from them exhibit changes in size and coloration. This paper is reviewed, and supplemented with reports of corresponding observations, by Schröder,[2] who in a previous article [3] showed that even nidification (in *Gracilaria stigmatella* F.) and habits of feeding, combined with changes in reproduction (in the beetle *Phratora vitellinoe* L.), can be changed and that these acquired characters are transmitted spontaneously from the third generation.

In 1907 and 1908 Paul Marchal [4] succeeded in transferring numerous specimens of *Lecanium corni* Bouché from the peach (*Amygdalus persica* Linn.) to the black locust (*Robinia pseudacacia* Linn.). Eggs hatched and larvæ developed on the new host plant, spreading out over the leaves in large numbers, and in the fall migrating from the leaves to the wood for hibernation. In the summer of 1908 the insects completed their development and had then the large size, deep coloration, and characteristic appearance of the insect described by Douglas as *L. robiniarum*, the attacks of which on the black locust had been severe in several European localities. This indicated that *L. robiniarum* was only a race of *L. corni*, resulting from individuals that had become transferred in some manner from the peach to the introduced American black locust. Dr. Marchal found great difficulty in reestablishing on the peach individuals of *L. corni* produced on the black locust.

There are other records showing the acquired adaptation of certain species to new host plants, similar to those here cited. The practical application of such phenomena, however, has, so far as can be ascertained, first been recognized by Dr. A. D. Hopkins (referred to on p. 189 of this article) and presented by him in concrete form.

In a paper prepared by M. Joseph Capus [5] on a nematode disease of peas in the Gironde and read by Paul Marchal at the session of July 10, 1918, of the French Academy of Agriculture there is a record of injury to peas by a fungus (*Fusarium vasinfectum* var. *pisi* van Hall, considered as the conidial form of *Necosmopora vasinfecta* E. F. Smith, accompanied by

[1] PICTET, Arnold. INFLUENCE DE L'ALIMENTATION ET DE L'HUMIDITÉ SUR LA VARIATION DES PAPILLONS. *In* Mém. Soc. Phys. et Hist. Nat. Genève, v. 35, fasc. 1, p. 45–127, pl. 2–5. 1905.

[2] SCHRÖDER, Chr. DIE LITERATUR ÜBER DIE FÄRBUNG DER INSEKTEN DES JAHRES 1905. *In* Ztschr. Wiss. Insektenbiol., Bd. 3, p. 162–164. 1907.

[3] ——— ÜBER EXPERIMENTALL ERZIELTE INSTINKTVARIATIONEN. *In* Verhandl. Deut. Zool. Gesell., Jahresversamml. 13, p. 158–166. 1903.

[4] MARCHAL, Paul. LE LECANIUM DU ROBINIA. Compt. Rend. Soc. Biol. [Paris], t. 65, p. 2–5. 1908.

[5] CAPUS, Joseph, and MARCHAL, Paul. SUR LA MALADIE VERMICULAIRE DES POIS DANS LA GIRONDE. *In* Compt. Rend. Acad. Agr. France, t. 4, no. 25, p. 712–716. 1918.

the nematode *Heterodera schachti* Schmidt). After pointing out the interdependence and relation of the two, M. Capus says:

> One might ask himself why this species, everywhere known for its injury to beets, does not establish itself on this plant in the Gironde and appears so abundantly on peas.

Following M. Capus's explanations of this phenomenon, Dr. Marchal observed:

> Among the very interesting facts pointed out by M. Capus in his note I wish to call attention to the following: That injury to beets by *Heterodera* in the Gironde is not constant, is rare. We should recall in this connection the observations, already old but interesting, of the Dutch naturalist Ritzema-Bos. He has shown that when nematodes multiply in course of years without interruption on the same host, biologic races are formed adapted to this host which later pass to other vegetation with greatest difficulty, even when these are of those preferred by the species. It must be, therefore, that, by virtue of the conditions of pea culture in the Gironde, a race of *Heterodera schachti* was formed especially adapted to peas and to the attack of which beets are resistant up to a certain point. There is no doubt that it will adapt itself to beets cultivated for a number of years in succession in the same soil infested with *H. schachti*.

The experiments conducted at the Gipsy Moth Laboratory[1] show that of the many plants tested a decided variation was found in regard to the susceptibility to attack by this insect. The plants were divided into four groups: I, favored species; II, favored food species after early stages; III, species on which a small proportion may develop; IV, species that are unfavored food. These results show that, although this insect has a wide variety of hosts on which it is capable of feeding, certain ones are selected in preference to others in the natural forests. As far as known, no observations have been reported showing whether or not several years' feeding on any particular host produces a strain which selects that in preference to others.

In a recent paper Dr. C. T. Brues[2] writes as follows (on p. 328–329):

> It has been claimed that the food habits may be modified experimentally, in that caterpillars reared on a strange plant (where they could be induced to select it) give rise to moths whose progeny more readily accept the new plant. It is very difficult to accept such evidence, at least as having any general application, without very clear and incontrovertible proof. If such transformations can occur so easily and become hereditary so quickly they should have entirely destroyed the coherent habits now existent, during the enormous period which has elapsed, for example, since the violet-feeding Argynnids were differentiated, since the holarctic and nearctic Vanessids have been separated, or while the world-wide Aristolochia-feeding Papilios were attaining their present distribution. That such a change has actually occurred in the case of other groups seems equally evident, although, as has been shown, we can more easily believe that they may have arisen through mutations in maternal instinct not incompatible with larval tastes and then only in extremely rare cases and confined to certain groups.

METHODS OF CONDUCTING EXPERIMENTS

In connection with the experiments by the writer several types of cages, the particular type determined by the amount of material handled

[1] MOSHER, F. H. FOOD PLANTS OF THE GIPSY MOTH IN AMERICA. U. S. Dept. Agr. Bul. 250, 39 p., 6 pl. 1915.

[2] BRUES, Charles T. THE SELECTION OF FOOD-PLANTS BY INSECTS, WITH SPECIAL REFERENCE TO LEPIDOPTEROUS LARVAE. In Amer. Nat., v. 54, no. 633, p. 313-332. 1920.

and the exact conditions required, have been used in confining the colonies of beetles. It is essential to duplicate as closely as possible the conditions in which the insects are found in nature.

For the larger logs and for experiments in which a large amount of material was used, an open wire insectary was constructed. This insectary is 40 feet long by 10 feet wide by 7 feet high. The foundation is of concrete, the side walls and top of 18-mesh galvanized wire screening, and over all a removable lattice-work roof was placed. This roof was adjusted to simulate shade conditions in the woods. It was removed in winter and replaced in summer. The floors were made of ashes to give good drainage. Cross partitions divided the insectary into seven compartments of different sizes. One room was entirely boarded in and roofed over. It was used for seasoning wood. Another was lined with cheesecloth, which was used for holding different cuts of wood under natural conditions until desired for use. In the other compartments were placed logs containing various species of insects. Where no danger is present of any infestation from the original host wood into cuts of different wood, it was possible to place several beetle species in the same compartment and continue their breeding in the same host from year to year. In this way forms such as Callidium in pine (*Pinus* spp.), *Neoclytus capraea* Say in ash (*Fraxinus* spp.), and *Cyllene pictus* Drury in hickory (*Hicoria* spp.) were placed together.

The smaller insects, especially those in twigs and branches, were confined in glass museum cylinders of various sizes. The tops were kept in place so that a very constant degree of humidity could be maintained. This cage was found to give best results for the development of the larvæ and, as no sand was needed, the adults were easily found in the cages. These jars were kept under a roof all the year in another insectary.

Each of these insects has a particular preference for a certain condition or seasonal cut of wood. Also in some species the adults require food before ovipositing, consisting of green bark from twigs, leaves, or fungus spores. In the latter case the pustule of the chestnut blight (*Endothea parasitica* (Murr.) P. J. and H. W. Anderson) was used. Again, some require much moisture, others rather dry surroundings. The determination of these factors sometimes delayed the successful continuance of a species for a year or more. When a new colony was collected from nature it was ascertained as nearly as possible when the tree died and the condition of the wood, also what degree of humidity was desirable. For instance, those insects naturally feeding in dead branches of a standing tree required drier conditions than those attacking branches fallen to the ground.

In order to meet these conditions, wood of the various species used was cut every month or every other month of the year and stored under different conditions. Part was placed in the dry shed for dry seasoning, part hung or stood up in the open-air cage for normal air seasoning, and

part laid on the ground in the wire cages for wet seasoning. The condition of the wood on which the insects were first found eliminated the use of certain of these periodic cuts and conditions of seasoning. However, the first-year adults were usually caged with the choice of many of these cuts and the one infested most heavily was considered as the optimum condition and used afterwards for continuation of the successive broods. The optimum cut could only be determined when sufficient material was given for the number of insects present, as an unfavorable cut may be attacked when the adults are confined on it without sufficient optimum material.

Wood used a month or two after being cut is spoken of as green or freshly cut material.

In many cases wood from several individual trees was used to avoid any possibility of offering an undesirable individual.

To illustrate the variation in optimum conditions of wood, several examples are given: *Callidium antennatum* Newm. requires wood dry-seasoned over winter; *Neoclytus capraea*, wood cut during the late winter with the inner bark still sappy; *Liopus alpha* Say in hickory, twigs cut in the early fall, air-seasoned for a while and then left on the ground over winter so that the inner bark sours somewhat. (This condition is brought about by the girdling habit of *Oncideres cingulata* Say.)

Several terms which may need explanation are used in reference to the species of host wood: *Primary host*, or *original host*, refers to the wood in which the insect is found in nature and first caged in these experiments; as *secondary host* is understood wood in which a colony has been successfully produced in the experiment, but it may or may not be recorded as a host in nature; an *unfavorable host* is one not recorded from nature and in which attempts to produce a colony have not been entirely successful.

All experiments conducted are here given, although a few have been unsuccessful or have given no results. Occasionally failure to continue a colony is recorded. In all cases an explanation can not be given. It may be because of an improper cut of wood or of a peculiarity of the individual host. In one case partial failure was due to a nematode parasite causing sterility of the females; in another, the parent insects were entangled in spider webs and killed before ovipositing.

Reference is made to larval transfers from one host to another. This is accomplished by making a smooth cell through the bark of the new host, partially filling it with frass from the larval mines of the original host, then placing the larva in this cell and finally tightly fixing a piece of bark over the cell. Such transfers do not injure the larva or affect its development. Many cases of transfer to the same host resulted in the survival of every larva.

These experiments were conducted at the Eastern Field Station, East Falls Church, Va., and all flight dates of the adults and times of cutting of the wood refer to this locality unless otherwise stated.

OUTLINE OF EXPERIMENTS ON EACH SPECIES

XYLOTRECHUS COLONUS. EXPERIMENT I

Xylotrechus colonus Fab. is found in nature in a wide variety of hosts. In fact, it feeds in nearly all hardwood deciduous trees of the eastern and central United States. It shows little or no preference for any exact condition of the wood, except that it will not attack perfectly seasoned material. The larvæ can be found in dying standing trees or in logs felled in any month of the year provided they still contain a certain amount of moisture.

The first flight of the year occurs in the last week of May or first week of June, reaching the maximum in about two weeks. A few adults emerge sporadically throughout the summer. From eggs deposited in June a few adults usually emerge in September, but the main brood remains as larvæ until the next spring. These fall adults have never oviposited under confinement.

The larvæ feed entirely beneath the bark, or in the bark if it is thick. The pupal cell is made in the outer sapwood or in the bark.

The wood of all species for this experiment was cut on April 15 unless otherwise stated. The colony was started by felling a red oak tree in March, 1914. The wood was attacked during that June, caged soon afterwards, and the colony has since been maintained in red oak. From the original oak form colonies were secured in hickory (Hicoria), chestnut (*Castanea dentata* (Marsh.) Borkh.), locust (*Robinia pseudacacia* Linn.), red maple (*Acer rubrum* Linn.), and ash (*Fraxinus* sp.), in the following manner:

QUERCUS. EXPERIMENT I.—During May, 1915, hickory logs were placed in the cage with oak intended to carry on the colony. Many adults were present, somewhat over 100, and the hickory as well as the oak was subsequently found infested.

In June, 1916, in the same cage stocked with oak for continuing the colony, chestnut and hickory wood was placed. There was again an overabundance of adults and all woods were infested.

In June, 1917, oak was placed in this cage to continue the colony and also ash, chestnut, locust, hickory, and red maple logs, all cut in February except the hickory, which was cut in April, 1917; extra pieces of chestnut and maple, cut in November, 1916, and September, 1916, respectively, were also placed in the cage. There was an abundance of adults. In July these logs were examined and it was found that the oak was heavily infested; the chestnut and hickory were lightly infested; the ash, maple, and locust had no infestation. This same year, 1917, adults were isolated on ash (I^5), maple (I^4), and locust (I^3) with results as described in later paragraphs.

In June, 1918, two pairs of adults from oak were caged on a collection of oak, hickory, ash, maple, and chestnut logs, all cut April 15, and of about equal size. Examination in July showed that the oak was heavily infested (over 50 larvæ present), the chestnut contained 10 larvæ, the hickory 7 larvæ, and the ash and the maple none.

At the same time a similar cage was prepared, and six pairs of adults were placed in it to test the influence of a greater number of beetles on the selection of hosts. The results showed the same relative proportion of infestation except that ash also was attacked. The maple was not infested.

In June, 1919, this experiment was repeated with the same conditions except that the hickory sticks were accidentally omitted. They were examined in July and the infestation was as follows: The woods in the cage of two pairs of adults contained 28 larvæ in oak, 22 in chestnut, and none in ash and maple; that of six pairs contained over 50 in oak, 19 in chestnut, and none in ash or maple.

HICORIA. EXPERIMENT I [1].—May, 1915, hickory logs were placed (as before described) in the oak cage with the wood intended to carry on the colony. They were infested and in subsequent years kept isolated and continued as the hickory form.

In June, 1916, oak was placed in this cage, together with the hickory to continue the colony, and was subsequently found heavily infested.

In June, 1917, together with the hickory for reinfestation, chestnut and locust were placed in the cage. An examination in July showed that the chestnut contained a few larvæ and the locust none.

In June, 1918, two pairs of adults were isolated in a cage containing oak, hickory, ash, chestnut, and maple, all cut April 15, and of equal size. The results showed that the hickory was heavily infested by over 50 larvæ, the oak contained 7 larvæ, the chestnut 1 larva, and the maple and ash none.

In June, 1919, selection tests and selection quantity tests were carried out with this strain. The quantities and cuts of wood were the same as before, except that oak was accidentally omitted. In one cage two pairs of adults were isolated, the resulting infestation being hickory 18 larvæ, chestnut 12 larvæ, maple and ash none. In another cage six pairs of adults were isolated, the resulting infestation being hickory over 50, chestnut 40, and maple and ash none.

CASTANEA. EXPERIMENT I [2].—In May, 1916, chestnut logs were placed (as before described) in the oak cage with the wood intended to carry on the colony. The wood was heavily infested, and these individuals have since been confined to chestnut.

In May, 1917, together with the chestnut, hickory was placed in this cage. The hickory was lightly infested.

In June, 1918, two pairs of adults were isolated in a cage containing oak, hickory, ash, chestnut, and maple, all cut April 15 and of equal

size. An examination in July showed that the oak and chestnut were equally well infested, the hickory contained one larva, and the maple and ash none.

ROBINIA. EXPERIMENT I [3].—In June, 1917, eight adults from oak were isolated on black locust cut in February, 1917. The adults laid eggs, but all died later.

In June, 1918, the experiment was repeated with 15 adults and wood cut April 15. Many young larvæ entered the bark, but by August 15 nearly all had died, and none lived to transform the next spring. The experiment was not repeated in 1919.

ACER RUBRUM. EXPERIMENT I [4].—In June, 1917, eight adults from oak were isolated on a piece of red maple cut February 1, 1917. A few larvæ lived and three adults (two males and one female) emerged in 1918. They were isolated in a cage containing oak, chestnut, hickory, ash, and maple, all cut April 15, but no infestation occurred in any wood. During August, 1918, twenty larvæ were transferred from oak to maple to continue the species in this host. A few adults emerged in 1919, and were recaged on maple to develop a larger colony, which will be continued several years before testing the selection again.

FRAXINUS. EXPERIMENT I [5].—In June, 1917, eight adults from oak were isolated on a piece of ash cut January 1, 1917. A fair infestation occurred, but the larvæ developed slowly, and in May and June, 1918, only seven adults (both sexes represented) emerged, while many larvæ were still in the wood. These adults were transferred to a cage containing oak, chestnut, hickory, ash, and maple, all cut April 15, but no infestation occurred in any wood. Larvæ were again transferred to ash, and a few adults emerged in 1919. These were recaged on ash, and several adults emerged in June, 1920, but failed to develop any larvæ in the new wood.

I, I^1, I^2, I^4.—During June, 1920, adults emerged from the oak, hickory, chestnut, and maple; strains and adults from all were recaged on the same wood and produced new colonies. No selection tests were made, and these strains will be continued for several years in the same wood before similar experiments are again attempted.

CONCLUSIONS

The original oak strain of *Xylotrechus colonus* shows a decided preference for a few woods, notably oak, chestnut, and hickory. Two years' trial failed to produce larvæ capable of completing their development in locust, while the ash and maple colonies were maintained with difficulty. In nature these woods (ash, maple, and locust) have been found containing thrifty colonies of this species.

Originally the oak strain showed little preference as between oak, hickory, and chestnut; yet, after several years, strains were developed in each wood that showed a growing preference for the given wood.

The number of insects present under identical conditions influences their selection of hosts. When few are present they concentrate on original or favored hosts; when more than can successfully oviposit on original hosts are present, less favored hosts are taken.

CYLLENE PICTUS, HICKORY HOST STRAIN. EXPERIMENT II

The larvæ of *Cyllene pictus* feed almost exclusively in hickory. A few specimens have been taken in wild grapevine (*Vitis* sp.), mulberry (*Morus rubra* Linn.), osage orange (*Toxylon pomiferum* Raf.), and hackberry (*Celtis occidentalis* Linn.), but such instances are rare and of very local occurrence. In one locality near Harrisburg, Pa., all except one of these unusual food plants have been recorded. This borer is found generally distributed east of the Mississippi River. The optimum condition of wood is that cut during the winter, preferably in January, and left lying on the ground. November cuts are sometimes attacked, but no wood is suitable unless the inner bark is still sappy. Sticks cut at the time of emergence are too green for attack.

The first emergence occurs about the middle of April and continues for three weeks. By September the larvæ are full grown and have constructed their pupal cells in the wood. They soon pupate, and in this stage they overwinter. The larvæ feed about equally beneath the bark and in the wood.

These experiments were started in April, 1915, when adults were found ovipositing on a hickory log cut during the winter at Falls Church, Va. The strain has since been continued in January and February cuts of this wood, and other host strains have been attempted with varying success in grape (*Vitis* sp.), locust (*Robinia pseudacacia*), ash (*Fraxinus* sp.), and mulberry (*Morus rubra*). Experiments were conducted as follows:

VITIS. EXPERIMENT II[1].—In April, 1917, a piece of grape, cut in January, was placed in the same cage with much hickory used for the continuation of the hickory form. This grape was not infested.

June 26, 1917, sixteen larvæ, about half grown, were transferred from hickory to grape cut in January. Nearly all these larvæ lived, and the following spring 12 adults emerged. They were isolated in a cage containing several pieces of grape and one of hickory, both cut in February. Examination in June showed the grape to be heavily infested while the hickory contained no larvæ.

In April, 1918, a large number of adults emerged from the grape. Two pairs were isolated in a cage containing one piece of grape 2 inches in diameter and 2 feet long, and one piece of hickory of the same size, both cut in January. Examination in July showed the grape to be very heavily infested, whereas the hickory contained only a few larvæ.

ROBINIA. EXPERIMENT II[2].—April 21, 1917, three females and two males from hickory were caged on a piece of locust cut a month previously. The females laid all their eggs on the locust and the young

larvæ bored through the bark, but by the middle of June all had died. June 15, twelve larvæ 5 mm. long were transferred to locust and these all died by July 11, when three more, over half grown, were transferred. These lived to construct pupal cells and pupated, but all the pupae died during the winter.

In April, 1918, five adults (three females and two males) from hickory were caged on locust cut during January, 1918. The females laid all their eggs, but only a few larvæ lived. These constructed pupal cells and pupated beneath the bark. In locust the larval mines are not normal, lying in almost all cases immediately beneath the bark instead of extending deep into the wood. About half the larvæ made pupal cells in the outer sapwood while the others pupated beneath the bark instead of, as normally, deep in the wood.

April 20, 1919, a total of six adults had emerged and two pairs were caged on pieces of locust and hickory cut in January, 1919. An examination July 16 showed no infestation in either.

FRAXINUS. EXPERIMENT II[3].—April 24, 1917, three females and two males were isolated on ash cut during January. The females laid all their eggs and the young larvæ bored through the bark, but all died before June 15. At this time fourteen larvæ 5 to 7 mm. long were transferred to the same ash, and all died within a month. July 15, five more, over half grown, were transferred. They mined extensively beneath the bark, but all died before the end of September without pupating.

July 24, 1918, fifteen larvæ, one-half to three-fourths matured, were transferred to ash cut in January.

April 21, 1919, a total of eight adults had emerged. One pair was caged on the January cut of ash, and two pairs were caged on January cuts of ash and hickory.

July 16 the wood was examined, but in no case was it infested.

MORUS. EXPERIMENT II[4].—April 29, 1918, three females and two males from hickory were caged on mulberry cut in January. The females laid eggs, and a very heavy infestation was secured. They developed normally and suffered little more than the normal rate of mortality experienced in hickory.

April 21, 1919, a total of 17 adults had emerged; two pairs were transferred to a cage containing two pieces of mulberry and one piece of hickory cut during January, 1919. In another cage containing the same quantity of wood four pairs of adults were transferred. In neither case was the quantity of mulberry sufficient to permit the development of all the larvæ. Each piece was 2 inches in diameter and 14 inches long.

July 16 the cages were examined, and that containing two pairs of adults was infested as follows: Hickory 6 larvæ, mulberry over 30; that containing four pairs, hickory 13 larvæ, mulberry very heavily infested, over 40.

SEASONED HICORIA. EXPERIMENT II[a].—Attempts were made in April, 1917, to develop a colony adapted to seasoned wood by caging two females and one male on wood cut in October and dry seasoned. Eggs were laid and larvæ entered the bark but developed very slowly, never entering the wood. Only three lived to pupate, and these made their pupal cells between the bark and wood. All three pupæ died during the winter.

In May, 1918, the experiment was repeated with three females and three males and wood cut in November, 1917. In the fall of 1918 a number of larvæ lived and pupated, but all were below normal size. Only a few adults emerged in April, 1919, and these were below normal size.

II[b].—Dr. A. D. Hopkins, in 1916, recorded a dying hickory tree heavily infested by *Cyllene pictus* with no evidence of primary injury from other causes.

This suggested that a strain capable of attacking living trees might be produced, and attempts were made to secure a colony in such a tree. A small hickory 3 inches in diameter was selected and entirely stripped of leaves August 11, 1916. April 30, 1917, it was again defoliated, and 80 adults were caged on it. The adults laid eggs and the young larvæ entered the bark, causing sap to flow from the wounds. However, all died after growing to 3 mm. in length.

In April, 1918, the tree was again defoliated, and 156 adults were caged on it. The same results were observed.

During both years the tree put out healthy foliage after artificial defoliation, but it died in August, 1917. In no case did the Cyllene larvæ live to mine more than ¼ inch beneath the bark.

QUERCUS. EXPERIMENT II[c].—In transferring adults during the spring of 1918 to new hickory wood to continue a large colony, a piece of oak was unintentionally left in the cage. This cage contained six large hickory logs 4 to 6 inches in diameter and 5 feet long. The oak log was 3 inches in diameter and 4 feet long.

During September, 1918, work of Cyllene was noticed on this piece of oak, and in the spring of 1919 it was separately caged. Five adults emerged in April—all very small, much below normal size.

Two females and one male were transferred to a cage containing only oak; one pair to a cage of oak and hickory. These cages were examined July 16. Neither wood of the selection test was infested, but the oak wood on which two pairs were caged contained a few very small larvæ.

II[st].—To test the influence of host selection on the condition of host. In April, 1918, two males and two females from hickory were isolated in a cage containing a piece of grape and a piece of hickory of equal size—the grape of optimum cut, January, 1918, the hickory less favorable, November, 1917. Examination in July showed the grape to be heavily infested while the hickory contained very few larvæ.

II[82].—To test the influence of an overabundance of adults and scarcity of the primary host on the host selection. Three pairs of adults from hickory were caged on a small piece of grape and a small piece of hickory (each 2 inches in diameter and 1 inch long), each cut during January, 1918. Examination in July showed both woods to be infested, the grape containing a few more larvæ than the hickory.

In 1920 no adults emerged.

II, II[4].—In 1920 only two strains were continued, those in hickory and those in mulberry. No attempt was made to reestablish the others that failed.

CONCLUSIONS

This species, although most commonly found in hickory, will readily adapt itself to several other plants, notably mulberry and grape, both of which are recorded as natural hosts.

In some unfavorable hosts, or in an optimum host in an unfavorable condition, the larvæ may become established, but the mortality is high and the progeny seem to be sterile.

After one year's feeding in a new host the larvæ may select that host in preference to others.

The selection of a host is influenced by the number of adults present and the quantity of the primary host, in that adults will prefer a secondary host to overinfesting the original host.

The selection of a host is influenced by the condition of the host, a favorable condition of secondary host being preferred to an unfavorable condition of the original host.

The optimum condition of any host capable of properly supporting growth of the larvæ is of very restricted limits.

CYLLENE PICTUS, GRAPE HOST STRAIN. EXPERIMENT III

This is the same species as previously discussed, having the same biological habits except that this host strain in grape (Vitis) was taken in nature at Hummelstown, Pa., in January, 1916, by J. N. Knull. Since then the colony has been continued at East Falls Church, Va., in grape cut in January or February. Other host strains have been produced and experiments conducted as follows:

HICORIA. EXPERIMENT III[1].—When these adults emerged from grape (May, 1916), three females and two males were isolated in a cage containing 10 pieces of grape and 1 piece of hickory cut in February, 1916. No eggs were laid on the hickory. April 17, 1917, two pairs of adults were isolated on hickory cut in January, 1917. Eggs were laid and the larvæ developed but not so rapidly as in the grape. June 15 they were under normal size. By September only two larvæ were alive. One of these pupated but died during the winter.

Many adults emerged from the grape in 1918 and five pairs were caged on three small pieces of grape 1½ inches in diameter and 2 feet long and one piece of hickory about the same size, both cut in January, 1917. In July they were examined, and both hickory and grape were heavily infested.

QUANTITY SELECTION. EXPERIMENT III[8].—To again test out the effects of host selection when an insufficient amount of wood is given than that required for the number of adults present, in April, 1918, two pairs from grape were caged on grape and hickory cut in January, 1918. The piece of grape was 2 inches in diameter and 1 foot long, the hickory 2 inches in diameter and 2 feet long. An examination in July showed both grape and hickory infested.

This experiment was repeated in April, 1919, using two pieces of grape and one piece of hickory, all of optimum cut and equal size. In one case 1 pair of adults was isolated, in another case 3 pairs were used. The wood on which 1 pair was caged contained 5 larvæ in hickory and 3 larvæ in grape; that on which three pairs were caged contained 4 larvæ in hickory and over 25 in grape.

The grape colony was not continued in 1920.

CONCLUSIONS

This host variety from nature had acquired a decided preference for grape.

The selection of a host is influenced by the quantity of wood present for a given number of adults, in that the adults will select a new host in preference to overinfesting the original host.

The tendency in this species in nature to confine itself to a certain host, either hickory or grape, is not as marked as in some other species.

CYLLENE PICTUS, HICKORY STRAIN II × GRAPE STRAIN III

In order to determine whether crossing of these two host strains would influence the progeny in the selection of the host, males and females were isolated from their pupal cells in the spring of 1917. April 17 three females from hickory and two males from grape were isolated in a cage containing hickory. Two females from grape and two males from hickory were isolated on grape. In neither case did mating occur as readily as when both sexes from the same host were paired. The sexes often approached each other and moved away before finally copulating.

Good infestations were secured in both cases. In April, 1918, one pair from hickory was caged on equal amounts of grape and hickory cut in January, 1918. Only the hickory was infested. Two females and two males from grape were isolated on the same amount of grape and hickory cut in January, 1918. Both woods were infested. These pieces were all 2 inches in diameter and 18 inches long.

CONCLUSIONS

This crossing of the two host forms had no influence on the selection of hosts.

The amount of wood and number of adults present influence the host selection, as shown when one female and two females were given the same amount of wood.

CYLLENE CRINICORNIS. EXPERIMENT XLI

Cyllene crinicornis Chev., found in the southwestern United States, is known to feed only in mesquite (*Prosopis juliflora* (Swartz) de C.) and occasionally on an allied legume, paloverde (*Parkinsonia microphylla* Torr.). In general its biology is similar to that of *C. pictus*, and it prefers the same conditions of wood. Adults begin emerging in the natural range during late February, and part of this generation emerges in September.

Mesquite infested with these larvæ was sent to Falls Church, Va., by T. E. Snyder from San Antonio, Tex., April 27, 1917. Adults emerged at Falls Church the following May and were caged on mesquite cut in March, 1918. A good infestation was secured and has since been continued on mesquite.

ROBINIA. EXPERIMENT XLI[1].—In May, 1918, two pairs were isolated on locust cut February, 1918. May 31 the females were dead, and the abdomens were dissected and found to contain eggs. Probably no eggs were laid, and in July no evidence of larval work could be found.

July 24, 1918, seven nearly matured larvæ were transferred from the mesquite to locust cut January, 1918. During May, 1919, five adults emerged. One pair was caged on locust cut in January, 1919, and one pair on both locust and mesquite. The mesquite was cut October, 1918.

July 16, 1919, these cages were examined, and the selection test showed that mesquite was not infested, whereas the locust contained several small larvæ. The cage containing only locust was lightly infested. None of these larvæ transformed in 1920.

CONCLUSIONS

The locust was such an unfavorable host that the adults would not oviposit on it, but larvæ may live and transform for one or two generations when forced to take it.

CALLIDIUM ANTENNATUM. EXPERIMENT IV

Some confusion exists as to the taxonomy of the blue species of Callidium allied to *Callidium antennatum*. A number of species have been described of questionable validity. Two species have been experimented with—*C. antennatum* and *C. janthinum* Lec. These two adults are easily separable, and their habits are also quite distinct. The

former, so far as the Forest Insect records are concerned, feeds only in pine (Pinus) and spruce (Picea), and for this discussion will be confined to the form occurring in the northeastern United States. It shows a decided preference for a certain condition of the host, requiring wood that has been cut in the early fall or winter and is well seasoned. When the inner bark is still sappy the insects will not normally make their attack.

Adults first appear about the middle of April, and the flight period continues about a month. One year is required to complete the life cycle. The larvæ feed beneath the bark until half grown, then enter the wood to construct a long pupal excavation, at the end of which the pupal cell is chambered off.

These experiments were started in December, 1916, when infested Virginia pine was caged. In 1916 and the following years the colony was continued in seasoned pine, and a form was also developed in spruce and freshly cut or green pine. Unsuccessful attempts were made to produce a juniper (Juniperus) strain. This insect has been reported as feeding in juniper (Juniperus) and maple (Acer).

JUNIPERUS. EXPERIMENT IV[1].—In April, 1916, juniper was placed in the cage together with pine; in addition, about 20 adults were isolated on a stick of juniper (both woods were cut in January, 1916). In neither case was the juniper attacked, and the females isolated on juniper failed to oviposit.

The same test was repeated in 1917 with juniper cut in October, and the same results were obtained. During June and July, 20 larvæ, from small to over half grown, were transferred to juniper. All finally died, some living a month. None increased in size before death.

PICEA. EXPERIMENT IV[2].—April 12, 1916, a piece of seasoned spruce was placed in the cage, together with the pine, for reinfestation. An examination in June showed only one larva in the stick, and this was far below the normal size of those in pine. By July 11 this larva had died. It is possible that more eggs were laid on the spruce but the larvæ died earlier.

June 29, 1916, nine larvæ about half grown were transferred to the same spruce wood. July 11, 1916, three larvæ were alive and 17 more were transferred. From these larval transfers 10 adults were secured in April, 1917. They were caged on four small pieces of seasoned spruce and a piece of seasoned pine placed in the cage for one week, both cut in October, 1916. Examination in July showed the spruce well infested, but only 4 larvæ were found in the piece of pine.

In 1918 the same experiment was repeated, four females and three males from spruce being caged on four sticks of spruce and one of pine cut in September, 1917. An examination in July showed that the spruce contained many larvæ, but none were found in the pine.

In 1919 this was again repeated, but the results showed that pine was infested while the spruce contained no larvæ. Both woods were of November cut, but it is not likely that this would have such a decided influence. However, six adults were isolated on spruce to continue the colony, and this wood was very lightly infested. No explanation can be offered as to the reason for this discrepancy from former results unless the spruce wood was in an unfavorable condition.

During the same season another cage was prepared of pine cut during November, 1918, and green spruce cut in April, 1919. The pine was heavily infested; the spruce contained no larvæ.

EXPERIMENT IV[2]. To test effects of condition of wood on selection.—April 23, 1920, one pair of adults from spruce was caged on a favorable cut of pine (November) and freshly cut spruce (April). When the woods were examined on July 5, 1920, the pine contained many larvæ while the spruce contained none. At the same time a pair was caged on November pine and November spruce, both optimum cuts. Several days later the female was found dead in the cage and had laid no eggs.

ACER. EXPERIMENT IV[3].—As maple has been reported as a host of this species, attempts were made in 1916 to start a colony in this wood. On July 6, six half-grown larvæ were transferred to a seasoned piece of wood, but by July 21 all but one had died and it was smaller than when transferred. This one died soon after. None of the larvæ fed on the maple.

GREEN PINE. EXPERIMENT IV[a].—In 1916 some of the wood used to carry this colony along was cut March 1, and consequently little seasoned. It was very unfavorable for the ovipositing of the adults, but some eggs were laid on the pieces. The larvæ developed slowly and at the time of pupation were below normal size. The adults secured in 1917 averaged about one-half normal size. They were caged again on wood cut in March, 1917. Adults were secured in 1918 and again caged on the same condition of wood, and a good infestation was secured. While the author was absent for a month from the field station in the summer of 1918 these larvæ were all killed by a fungus. The sticks were on the ground and so were caught in a period of rainy weather and were water soaked.

EXPERIMENT IV, IV[2].—In 1920 the pine and spruce strains were continued in the same wood.

CONCLUSIONS

The pine form shows a decided preference for that host.

It can live in spruce and then shows a decided preference for that host.

It will not live in juniper or maple.

In producing a new host strain a high mortality occurs in the young larvæ.

A colony can be produced in a host which is in an unfavorable condition, but the resulting adults are below normal size. Owing to the failure to continue the colony it can not be stated whether or not such a strain would show preference for the new condition of the host by selecting it voluntarily.

CALLIDIUM JANTHINUM. EXPERIMENT V

Under *Callidium antennatum* reference was made to *C. janthinum* Lec. It is distinguishable from the former by its smaller size, shining surface, and bluish green color of the adult, by the fact that the larva feeds only in juniper, and that the adults emerge about four weeks later in the spring. It requires wood which was cut during the late fall and which has not seasoned in contact with the ground. It will oviposit in greener wood than *C. antennatum* although the inner bark should not be sappy.

The first flight occurs during the first to third week in May and continues about two weeks. One year is required to complete the development. The larvæ feed beneath the bark until half grown, then excavate long pupal chambers, the ends of which are plugged off for the transformation cell.

These experiments were started with a lot of infested juniper branches from Hummelstown, Pa., collected by J. N. Knull in April, 1916. May 2, six adults emerged and were caged on juniper cut in April and rapidly dried in the house. Since then the colony has been continued each year in September and November cuts of juniper, which are preferred.

PINUS. EXPERIMENT V[1].—May, 1917, two pairs were isolated on pine cut in November, 1916. A few eggs were laid from which larvæ hatched and entered the bark. By July 10 all had died. The same test was repeated in 1918 with similar results.

CONCLUSIONS

This species shows a decided preference for juniper and will not develop in pine from early stages. Larval transfers to pine with nearly matured larvæ were not made.

CALLIDIUM ANTENNATUM AND C. JANTHINUM. V×IV; IV×V

Because of the taxonomic confusion between these species (cited previously) and with the idea that a crossing of these two forms might possibly influence the selection of a host, attempts were made to cross the species.

V×IV.—In May, 1917, four females of the juniper form were crossed with two males of the pine form and were caged on juniper. Both species had been previously isolated from the pupal cells to avoid all possibility of mating. These insects immediately mated, and the eggs were laid on the juniper from which a good infestation was secured.

April 17, 1918, the first adult emerged from this cross. The second adult emerged April 25. May 3 and 4 eight adults emerged. All the 1918 adults resembled the juniper form in color. These adults were all isolated in a cage containing juniper and pine cut in November, 1917. An examination in July showed only the juniper to be infested, but by a very light brood. During the remainder of the summer all died except three larvæ which constructed pupal cells. May 5, 1919, two males and one female emerged and one pair was caged on juniper cut in November. A light infestation occurred. May 12, 1920, five adults emerged and were recaged on juniper. The selection of pine and juniper was not again tested.

IV×V.—These same species were mated in 1917 by making the reciprocal cross (males from juniper and females from pine) and caged on pine. The sexes did not mate readily, not noticing one another for some hours after being caged together. However, several matings finally took place and eggs were laid on the pine. Young larvæ developed but all died later. The same experiment was repeated in 1918 with the same results. Conditions were similar to those in the previous experiment.

CONCLUSIONS

The crossing did not influence the selection of a host in the first generation of resulting adults.

These two forms, even though they may be crossed successfully, should be regarded as distinct species based on adult characters and biological differences.

The successful cross-mating produced progeny in the first generation that emerged over the period of emergence of both parents—a few early when the pine form emerges, the remainder some two weeks later when the juniper form appears. In later years they emerge as the juniper form.

The juniper color pattern of the adults is dominant.

HYLOTRUPES LIGNEUS, JUNIPER FORM. EXPERIMENT VIII

The adult forms generally included under *Hylotrupes ligneus* Fab. show a great variation of color patterns. Many of these varieties have been described as distinct species by Col. T. L. Casey. In the experiments conducted all color varieties, however, have been kept distinct only by the host in which they were found in nature and not by the color variations. The experiments were primarily conducted to test these variations in color patterns, but certain results bearing on the host-selection principle were obtained and are here described.

Hylotrupes ligneus, juniper form, has a wide selection of hosts. Specimens in the Forest Insect Collection of the Bureau of Entomology have been recorded from all genera of coniferous trees indigenous to North America. It uniformly prefers wood that has not seasoned a great deal.

Later winter or fall cuts in which the inner bark has remained sappy are most suitable. Species of wood which season more slowly, due to thick bark, must be cut earlier.

The time of first emergence varies greatly with locality, but the species is everywhere one of the first cerambycid beetles to fly in the spring. The larvæ in all cases feed immediately beneath the bark, only entering the sapwood in late summer to make a shallow pupal cell. Pupation and transformation to the adult usually take place in the fall.

The present experiment was started at Kanawha Station, W. Va. Dr. Hopkins felled a juniper (Juniperus) in October, 1914. This tree was infested the following spring and shipped to East Falls Church, Va. The colony has since been continued in juniper and one host strain has been produced in Douglas fir (Pseudotsuga). This particular color variety had never been recorded from Douglas fir.

PSEUDOTSUGA. EXPERIMENT VIII[1].—April 11, 1917, three females and two males were isolated on a piece of Douglas fir cut April 1. Eggs were laid and young larvæ entered the bark, but many died during the summer and only two constructed pupal cells. One adult was secured next spring. The fact that this wood was too green and that it seasons very slowly may have caused a higher mortality than would otherwise have occurred.

May 29, 1917, twelve larvæ and June 15, nine larvæ were transferred to this host, the wood then being better seasoned. March 1, 1918, five adults—four females and one male—were removed from pupal cells. The remainder of the larvæ had died. Two females and one male were used to continue the colony by caging on Douglas fir, cut in October, 1917. A good infestation was secured. A piece of juniper cut during January, 1918, had also been placed in this cage but was not infested. In January, 1919, four adults were removed from the logs—three males and one female. The remainder had all died and these were very weak and below normal size. One pair was recaged on juniper cut in January and Pseudotsuga cut in November. July 30, 1919, the sticks were examined, but no infestation was found in either wood.

CONCLUSIONS

This juniper form of *Hylotrupes ligneus*, after feeding part of a year in a new host, showed a preference for the new host.

A high percentage of mortality occurred in producing the new host strain, which finally died out.

HYLOTRUPES LIGNEUS, PSEUDOTSUGA FORM. EXPERIMENT XXXV

This form of *Hylotrupes ligneus* is much darker and more hairy than the preceding. It has been recorded only from Douglas fir. Its biology is essentially similar to that of the juniper form except that the adults emerge somewhat later. It is known from the Rocky Mountain region.

The colony was started from a small tree collected at Colorado Springs, Colo., and shipped to Falls Church. This tree had been killed by Scolytus in the fall of 1916 and infested by Hylotrupes in the spring of 1917.

In 1918 adults did not emerge until May and were caged on Douglas fir to continue the colony. They were recaged on Douglas fir in 1919, but all the larvæ died from a fungus attacking the bark.

HYLOTRUPES LIGNEUS, PSEUDOTSUGA STRAIN VIII [1] AND PSEUDOTSUGA STRAIN XXXV

Two females from VIII [1] (the juniper form in Douglas fir) were held in a cool cellar until adults of this XXXV variety emerged. May 29, 1918, they were caged on Pseudotsuga with two males from the true Douglas fir form (XXXV). The two sexes absolutely avoided each other and were never observed to mate. The females died without laying eggs. Many attempts were also made to mate the original juniper form with the Douglas fir form but without success.

HYLOTRUPES LIGNEUS, SEQUOIA FORM. EXPERIMENT XL

The form of *Hylotrupes ligneus* occurring in sequoia is slightly larger but otherwise resembles that in juniper very closely, although the specimens reared in the experiments show a much greater variety of color pattern than do those from juniper.

April 2, 1918, a large series of these adults were removed from their pupal cells in *Sequoia sempervirens* (Lamb.) Endl. and isolated in small vials by F. B. Herbert at Laurel, Calif. April 13, 1918, they were received at Falls Church, Va.

JUNIPERUS. EXPERIMENT XL[1].—Three prominent color forms were paired and each was caged on a piece of juniper cut in January, 1918, since no sequoia was on hand. They all oviposited, but about half of the larvæ died by July. The remainder made pupal cells and emerged. The strain has since been continued in juniper.

PSEUDOTSUGA. EXPERIMENT XL[2].—April 20 one pair was caged on a piece of Douglas fir cut in October, 1917. Eggs were laid and a better infestation secured than with the juniper form (VIII). All larvæ died and no adults were secured in the spring of 1919. A fungus growth under the bark was responsible in a large measure.

HYLOTRUPES LIGNEUS, JUNIPERUS STRAIN VIII × SEQUOIA STRAIN XL

April 13 several males from redwood (XL) were separately caged with females from juniper (VIII) held over in a cool cellar since they were isolated from the cells. One of these males mated with two females (first and third tried) immediately on being isolated with them. This same male would not mate with the second female tried, nor would any males of XL mate with females of VIII. Many juniper (VIII) males

were isolated with redwood (XL) females, but in no case did copulation take place.

The females of the juniper form mated with males of the sequoia form were caged on juniper cut in January, 1918, and good infestations were secured.

CONCLUSIONS ON THE ENTIRE HYLOTRUPES LIGNEUS GROUP

The experiments on the *Hylotrupes ligneus* group, as mentioned above, were conducted primarily for the study of its color variation, and not a great deal of attention was devoted to the host-selection principle. The experiments cited show that among all the color varieties of this group there are probably two good species, the darker and more hairy Douglas fir form representing one species and all the other forms another. These two species absolutely refused to mate, but the varieties from sequoia and juniper were successfully crossed.

NEOCLYTUS CAPRAEA. EXPERIMENT VI

Neoclytus capraea is known to inhabit the eastern and central western United States, extending its range south and west into Arizona. It has been recorded from only two hosts, ash (Fraxinus) and white oak (*Quercus alba* of the Rocky Mountains). In the eastern United States it has never been found in oak. The condition of the wood necessary for oviposition by these beetles must be exactly right. It must have been freshly cut and the inner bark must be still moist and sappy. Should this inner bark be slightly dried the females will not oviposit on it unless forced to do so. Logs cut about two months before the flight period are preferred to older cuts or those cut during flight. Trees cut as early as November 15 are sometimes infested, but not commonly.

The adults fly very early in the spring in this locality (Falls Church, Va.), about the last week in March and the first two weeks of April. The larvæ feed chiefly in the wood proper. Mining beneath the bark for a short time, they then enter the sapwood and later the outer heartwood, extensively honeycombing it. Pupation and transformation to the adult take place in the early fall.

VI.—March 26, 1915, twelve adults were taken as they emerged from an ash log and were caged on freshly cut wood. A good infestation was secured, and the colony has since been continued in ash.

In the spring of 1919 no adults emerged. All the larvæ remained over as larvæ in their pupal cells until the fall of 1919, when they transformed to adults and emerged in 1920. No explanation for this can be offered unless the logs were too moist in the early part of the summer so that the larvæ did not develop properly. Excess humidity or excessive desiccation have both been found to produce retardation in development of larvæ in small isolated cages. This insect is one of the most regular of those reared, in the time of emergence and development of

the broods. Attempts have been made to start colonies in hickory and white oak.

HICORIA. EXPERIMENT VI [1].—March 31, 1917, seven adults (four females and three males) from ash were isolated on hickory cut February 1, 1917. No infestation occurred. May 31 of the same year fifteen larvæ, 2 to 4 mm. long, were transferred to hickory. Again on June 15, seven larvæ, 4 mm. long, were transferred to the same piece of wood. July 11 one larva was living and five more, over half grown, were transferred.

April 6, 1918, three adults (two males and one female) emerged from the hickory. They were caged on hickory and ash cut January, 1918. These adults were very weak and inactive, not at all characteristic of normal adults.

An examination in July showed neither wood to be infested.

July 24, 1918, twenty larvæ, one-half to three-fourths grown, were transferred from ash to hickory cut April 15, 1918.

April 11, 1919, one female emerged, one adult had died in its pupal cell, and the remainder of the larvæ had died before pupating. This female was mated with a male from ash and caged on hickory and ash of optimum cuts. An examination in July showed no infestation in the hickory, but the ash contained a few larvæ. These died later in the summer.

QUERCUS ALBA. EXPERIMENT VI [2].—April 1, 1917, four pairs of adults from ash were isolated on white oak cut in March, 1917. Eggs were laid on the wood, and the small larvæ bored through the bark, but all died before May 31. On this date fifteen larvæ, 2 to 4 mm. long, were transferred to white oak. July 11 one larva was living. September 17 all were dead.

In April, 1918, three pairs were caged on wood cut in January, 1918. July 18 many larvæ were still alive but under size. Several lived to pupate, but all died before the following spring.

SEASONING. EXPERIMENT VI.[a]—April 4, 1917, four pairs of adults were isolated on ash cut September 1, 1916, and white oak cut in March. The females laid eggs on the white oak, but the larvæ did not live. On May 31 neither wood contained larvæ.

CONCLUSIONS

The foregoing experiments show that this species feeding in ash (Fraxinus) has become decidedly accustomed to that host. Several attempts, both by oviposition and larval transfers, to produce strains in *Quercus alba* Linn. and Hicoria have resulted in failure. In Hicoria the few adults secured were incapable of continuing the colony, and in both woods a high or total larval mortality occurred.

Even with this decided preference for a host, the adults laid eggs on a new host rather than on an unfavorable cut of the normal host.

MOLORCHUS BIMACULATUS. EXPERIMENTS IX, X, AND XXXVI

Two forms included under *Molorchus bimaculatus* Say have been caged in these experiments, a large form from hackberry (*Celtis occidentalis* Linn.), and a smaller form from dogwood (*Cornus florida* Linn.) and maple (Acer). They both are found throughout the eastern half of the United States. From the observations on the biology of these two forms they are regarded by the writer as distinct species. Both forms prefer early fall cuts of wood, but the Celtis form requires much drier seasoned material.

MOLORCHUS BIMACULATUS, CORNUS FORM. EXPERIMENT IX

The Cornus form feeds in a great variety of eastern hardwoods. It has been reared from Hicoria, Acer, Juglans, Quercus, Liriodendron, Cornus, Cercis, and Castanea. The larvæ feed beneath the bark, making a long, curved pupal cell in the wood. By September they have transformed to adults, which emerge early in May at Falls Church, Va. The flight is very regular, nearly all emerging at the same time. The adults are much smaller than those of the hackberry form.

In May, 1916, adults were reared from dogwood collected at Falls Church, Va. They were recaged on dogwood cut in April, but a poor infestation was secured from which only five adults emerged in 1917. These five adults were caged on September and November cuts of dogwood and redbud (*Cercis canadensis* Linn.). A good infestation occurred in the dogwood, but no larvæ were found in redbud.

May 1, 1917, five adults were isolated in a cage containing November cuts of dogwood and maple. The maple was not infested, but many larvæ were found in the dogwood.

In April, 1918, 1919, and 1920, the colony was continued only in dogwood. No selection tests were made.

MOLORCHUS BIMACULATUS, ACER FORM. EXPERIMENT XXXVI

This form in all respects is similar to the dogwood variety IX.

Infested limbs collected at Falls Church, Va., were caged in the summer of 1916.

May 1, 1917, five adults were caged on branches of maple and dogwood cut in September and November. The maple was infested but no larvæ entered the dogwood.

In 1918 many adults emerged from the maple and were recaged on October cuts of maple and dogwood. Eggs were laid on the maple, but the cage unfortunately was overlooked and became so dry that none of the eggs hatched.

MOLORCHUS BIMACULATUS, CELTIS FORM. EXPERIMENT X

The form in hackberry, in which the adults are much larger, has been reared only from this host. The larvæ feed as in the dogwood or maple

forms, but only about half the brood emerges at the end of the first year, the remainder going over in the larval stage to the following season. The adults emerge about a month earlier, April 1 to 10.

Infested hackberry branches from Hummelstown, Pa., were collected and sent to Falls Church, Va., in December, 1915, by J. N. Knull.

In April, 1916, 20 adults were caged on January cuts of hackberry, dogwood, and redbud, but only the hackberry was attacked.

In April, 1917, eight adults were isolated on sticks of redbud, dogwood, and maple, all cut in September and November. No eggs were laid in any of these woods. The form has since been continued in hackberry.

CONCLUSIONS

A very decided predilection for the original host is exhibited by the host strains of this species. It is not surprising in the case of the hackberry form, as this is the only host from which it has been found. However, this form would not even lay eggs on any hosts other than the original. In the dogwood strain adults were not isolated on maple alone, nor were adults of the maple strain isolated on dogwood alone. If this had been done, it is very likely that infestations would have resulted.

NEOCLYTUS ERYTHROCEPHALUS. EXPERIMENTS XI, XII, AND XIII

The adult and larva of *Neoclytus erythrocephalus* Fab. are quite different from those of *Neoclytus capraea*, but the range and habits are much the same. The species attacks wood in a greater variety of conditions, but the most favorable condition is an early spring cut. It has been collected in almost all eastern hardwoods.

The first flight occurs at Falls Church, Va., in late May or early June; consequently, that the wood may be sappy for infestation it must be cut during April. The species overwinters in the larva stage, pupation not taking place until early April. Farther south two or more generations occur each season.

Three host strains were collected in nature and experimented with.

NEOCLYTUS ERYTHROCEPHALUS, HICORIA FORM. EXPERIMENT XI

June 9, 1916, adults emerging from hickory at Falls Church, Va., were recaged on wood cut in late March. A good infestation was secured.

June 8, 1917, the colony was continued in April cuts of hickory. Two pairs were isolated in a cage containing hickory and redbud cut in April and dogwood and tulip (*Liriodendron tulipifera* Linn.) cut in May. In July an examination showed hickory to be the only wood infested.

May 23, 1918, two pairs were isolated on hickory, dogwood, and redbud cut on April 15. When examined on July 18 hickory was found to be lightly infested, dogwood heavily, and the redbud contained no larvæ.

May 24, 1918, six pairs of adults were caged on two pieces of hickory and one of dogwood, cut April 15, of the same size as those of the

experiment of May 23, 1918. When examined on July 18, both woods were heavily infested. Redbud was unintentionally omitted.

This experiment was repeated in 1919, two pieces of hickory, one of dogwood, and one of redbud being used. Two cages were prepared; in one, a single pair was isolated, the resulting infestation being, hickory heavily infested, dogwood and redbud uninfested; in the other cage three pairs were isolated, the resulting infestation being, hickory and dogwood both heavily infested, redbud uninfested.

NEOCLYTUS ERYTHROCEPHALUS, CORNUS FORM. EXPERIMENT XII

June 13 to 15, 1916, adults emerging from dogwood at Falls Church, Va., were recaged on this wood cut in April, 1916. A good infestation was secured. June, 1917, the colony was continued in dogwood and two pairs of adults were isolated in a cage containing dogwood and tulip cut May 30 and hickory and redbud cut April 18.

In July it was found that both redbud and dogwood contained few larvæ while hickory and tulip contained none.

For some unknown reason the larvæ continued in dogwood did not develop very well, and in 1918 only one female emerged. May 25, 1918, this female was mated with a male from hickory and isolated in a cage containing dogwood, hickory, and redbud cut April 15.

July 18, 1918, the dogwood was heavily infested, the redbud lightly, and the hickory contained one larva.

In June, 1919, one pair was caged on pieces of dogwood, redbud, and hickory. An examination in July showed dogwood to be very heavily infested, the redbud and hickory containing seven and six larvæ, respectively.

NEOCLYTUS ERYTHROCEPHALUS, CERCIS FORM. EXPERIMENT XIII

Redbud infested with this species was collected at Hummelstown, Pa., by J. N. Knull and sent to Falls Church, Va., in April, 1916. Adults emerged in June and the colony was continued in redbud. June, 1917, the colony was again continued in redbud, and two pairs of adults were isolated in redbud and hickory cut in April and tulip and dogwood cut in May.

An examination in July showed the redbud to be heavily infested; the dogwood and hickory contained several larvæ, and the tulip none.

In May, 1918, two pairs were again caged on redbud, dogwood, and hickory, all cut April 15. In July it was found that the redbud and the dogwood were heavily infested while the hickory contained but three larvæ.

The same experiment was repeated in 1919, and the results showed the redbud to contain eight larvæ, the dogwood five, and the hickory two.

The selection tests of 1917 were all carried out with the same quantity of wood; in each case the pieces were 1¼ inches in diameter and 1 foot long. Each cage contained two pieces of the wood from which the

adults emerged and only one each of the others. This amount of the original host for two females was considered sufficient for oviposition without bringing in the quantity factor.

These adults are extremely active and run rapidly over logs when ovipositing in nature. They have very long hind legs. It was noticed that in the glass cylinder used for cages in 1918 these long legs were a disadvantage. The adults could not get a foothold on the glass and had difficulty in climbing up on the wood from the glass surface. They crawled awkwardly about and when coming in contact with any stick maneuvered until they managed to get on it. Such conditions may have influenced the wood selected, as the adults could only with difficulty go from one stick to another. In 1919 wire boxes were used, the wood lying flat on the bottom. In 1920 only ash and dogwood strains were continued.

CONCLUSIONS

These experiments up to 1919 did not seem to show results in any definite direction. Selections of the various host strains occasionally gave results in conformity with those generally obtained, while again just opposite results were recorded.

The experiments of 1919 showed results in closer conformity to those of other species. This may have been due to the different method of caging, which gave the adults more opportunity to move about and select the host.

LIOPUS ALPHA. EXPERIMENTS XXV AND XXX

Two color forms of *Liopus alpha* have been experimented with, a brown form from sumac (Rhus) and a gray form from hickory (Hicoria). These color forms are very distinct and easy to recognize as adults. They are not known from any other hosts. The sumac form has been collected throughout the eastern United States and as far west as the Rocky Mountains. The hickory form follows the range of the hickory trees.

The adults fly in late May and continue flying through June at Falls Church, Va. One year is required to complete the life cycle. The larvæ feed beneath the bark and pupate in the wood. They are found only in small branches.

LIOPUS ALPHA, RHUS FORM. EXPERIMENT XXV

The sumac form prefers branches cut in the early fall and dried standing in the air, although it will attack later cuts, provided they have dried considerably.

April 26, 1916, Mr. Champlain sent from Long Island, N. Y., a lot of infested sumac twigs which were caged at Falls Church, Va. In June the first adults emerged, and 20 were caged on sumac cut in November, 1915. Into the same cage were placed chestnut, hickory, and wild cherry twigs cut during the winter, but none of these latter woods were infested. Since then it has been continued in sumac.

CASTANEA. EXPERIMENT XXV[1].—September 20, 1916, fifteen larvæ, one-half to nearly full grown, were transferred from sumac to chestnut cut during March. July 10, 1917, one adult emerged, the only one from these transfers.

HICORIA. EXPERIMENT XXV[2].—July 25, 1916, eleven larvæ about half grown were transferred from sumac to hickory. August 9, eleven more were transferred. The larvæ seemed to do quite well and by winter many had made pupal cells.

During June, 1917, twelve adults emerged and were caged on pieces of hickory cut the preceding June, August, April, and February. No infestation occurred in any of the wood. The cage accidentally dried for a two-week period while the adults were ovipositing and this may account for the failure of infestation, as they require considerable moisture.

In June, 1917, adults from sumac were isolated in various cuts of hickory but no infestation occurred.

July 23, 1917, twenty-seven larvæ were transferred from sumac to hickory cut in September, 1916, and March, 1917. The larvæ did well and the following May and June 10 adults were reared and caged on hickory sticks cut in September, 1917. On several of these sticks bands of thin outer bark of sumac were tied.

The adults oviposited only on those sticks and at those places where the sumac bark was tied. July 30 they had not yet bored beneath the hickory bark proper, but by fall nearly all had entered the bark. Only one larva transformed to an adult in the summer of 1919. One adult emerged in 1920. Several larvæ did not transform but continued feeding beneath the bark during the summer of 1919.

LIOPUS ALPHA, HICORIA FORM. EXPERIMENT XXX

The Hicoria form was not successfully continued in confinement until the summer of 1917. It requires wood cut in August, dried in the air for a month or so, and then placed on damp earth over winter. In addition the adults must be well fed on fungus spores (*Endothea parasitica* was used) before they will oviposit.

It was again continued in hickory in 1918, 1919, and 1920. During June, 1919, many adults were caged on sumac branches and eggs were deposited. Three larvæ lived to construct mines under the bark, but these died before November.

CONCLUSIONS

From the foregoing experiments and the fact that each of these two color forms has been taken only in the host given, it is evident that each has become restricted to that host and shows a strong predilection for it. Even after having fed for one year in a new host (Hicoria) adults developing from them showed a preference, in their oviposition, for that part of the hickory twig surrounded by Rhus bark. A fairly high mortality of larvæ occurred after transfer to the new host.

HYPERPLATYS MACULATUS. EXPERIMENTS XXVI, XXVIII, AND XXIX

Hyperplatys maculatus Hald. occurs throughout the eastern United States and west through the Rocky Mountain region. Two very similar species have been described, *H. maculatus* Hald. and *H. aspersus* Say, but the distinction is not drawn here, as each has many variations in color and maculation. It feeds on a great variety of hardwood deciduous trees. Probably any wood is attacked, provided it is in the proper condition for infestation. Smaller twigs and branches are usually preferred. Those that have died during the fall and lain on the ground so that a certain amount of fermentation has taken place in the bark give the optimum condition.

The larvæ feed entirely beneath or in the bark, only entering the sapwood to make a very shallow pupal cell. Adults fly in the early summer, late May, and early June. Two distinct variations occur in the length of the seasonal history. One form takes an entire year to complete the development, only one generation appearing each year. Another matures from one-half to three-fourths of the brood in August and September, the adults emerging and infesting new wood. This may be a basis on which to separate the two confused species. Four host strains have been experimented with, chestnut (*Castanea dentata*), gooseberry (Ribes), dogwood (*Cornus florida* Linn.), and yellow poplar (*Liriodendron tulipifera* Linn.).

HYPERPLATYS MACULATUS, LIRIODENDRON HOST STRAIN. EXPERIMENT XXVIII

The colony was started by collecting infested tulip branches in November, 1916, at Falls Church, Va. The following June adults emerged and were isolated in a cage containing yellow poplar, maple, dogwood, chestnut, and gooseberry cut in the fall of 1916. The original host, yellow poplar, was well infested, and a few larvæ were found in gooseberry, but no other woods were attacked. In 1918, 1919, and 1920 the colony was continued in yellow poplar; the selection was not repeated.

Only one generation of this form occurs each year.

CASTANEA. EXPERIMENT XXVIII[1].—June 4, 1917, ten adults from yellow poplar were caged on chestnut cut in November, 1916. A very good infestation was secured, forty-five adults emerging in 1918. Eight of these adults were isolated in a cage containing yellow poplar and chestnut cut in November, 1917.

Examination in August showed the yellow poplar to be heavily infested, while no larvæ were present in the chestnut.

June 1, 1919, two pairs from yellow poplar were isolated on chestnut, and in 1920 thirty-six adults emerged. Ten were caged on optimum cuts of chestnut, and the yellow poplar was heavily infested.

HYPERPLATYS MACULATUS, RIBES HOST STRAIN. EXPERIMENT XXVI

This colony was started in December, 1915, with infested gooseberry stems sent to Falls Church, Va., from Colorado Springs, Colo., by G.

Hofer. It has since been continued in gooseberry cut at Colorado Springs in the fall and shipped to Falls Church; in addition, several other host strains were produced. Only one generation of adults occurs each year.

May 20 to June 10, 1916, adults emerged and 47 were isolated in a cage containing gooseberry, chestnut, and wild cherry, all cut in the preceding fall. The gooseberry stems were heavily infested, a few larvæ were present in the wild cherry, but none were found in the chestnut. The colony has since been continued in gooseberry.

PRUNUS. EXPERIMENT XXVI [1].—The infested wild cherry twigs (described above) were caged separately, and in June, 1917, four adults emerged. These were caged again on a fall cut of wild cherry. The infestation was not very good, and only six adults were secured in 1918; these were recaged on the same wood, but no infestation occurred.

CASTANEA. EXPERIMENT XXVI[2].—As previously stated, the chestnut sticks were not infested in 1916 when caged with gooseberry. In June, 1917, nine adults were isolated on chestnut cut in November, 1916, and a good infestation was secured. June, 1918, nine adults emerged and were isolated in a cage containing chestnut and gooseberrry cut in November, 1917. Later examination showed only the gooseberry to be infested.

LIRIODENDRON. EXPERIMENT XXVI[3].—June 5, 1917, eight adults from gooseberry were isolated on tulip cut in November, 1916. Five adults emerged from these sticks in 1918 and were isolated in a cage containing tulip and gooseberry cut in November, 1917. Neither wood was infested.

HYPERPLATYS MACULATUS, CASTANEA HOST STRAIN. EXPERIMENT XXIX

In April, 1916, at Falls Church, Va., branches of chestnut (Castanea) containing larvæ in the pupal cells were collected and caged. Some of the adults emerging in June were isolated with chestnut cut in March, 1916, and the others isolated in a cage containing chestnut and dogwood (Cornus) branches cut in March, 1916. Those isolated on chestnut alone attacked this wood although it was a late cut. Those isolated on the two woods infested both, but the dogwood more heavily. Nothing more was done with the chestnut form. Many adults emerged that fall.

CORNUS. EXPERIMENT XXIX[1].—The dogwood sticks were then caged separately and adults secured in September, 1916, and more of them in June, 1917. Those emerging during the latter period were recaged on August and November cuts of dogwood, but no infestation occurred.

CONCLUSIONS

In *Hyperplatys maculatus* host selection occurs to a certain degree; but this beetle behaves differently from most of the other species tested. Thus the tulip form (experiment XXVIII) in 1917 chiefly selected the same host, but it also oviposited on gooseberry. This gooseberry colony, however, was weak, and a high mortality in larvæ occurred.

Furthermore, although not selecting chestnut when the other host was present, they produced a good colony when isolated on it; but in 1918 these adults again selected tulip in preference to chestnut. The same was true with the original gooseberry form which was transferred to chestnut (experiment XXVI [2]), for in 1918 it returned to gooseberry in preference to chestnut.

SUMMARY OF RESULTS

(1) In practically all the species experimented with the adults show a marked predilection for the host in which they have fed as larvæ, provided they are not deterred by other factors, such as the unfavorable condition or the small quantity of the host.

(2) There is considerable variation in the degree of preference for the original host, as between different species. Thus—

(*a*) Certain species are capable of living in only one genus or species of plant, which consequently they select.

(*b*) Certain species, chiefly those living in nature in several hosts, can be forced to adopt a new host.

(*c*) Certain species, chiefly those feeding in nature in a great variety of plants, show little discrimination in the selection of hosts.

(*d*) Certain species feeding in nature in a great variety of hosts often show a preference for a few of these.

(3) In forced transference of individual adults of a species to a new host, a high mortality of the broods usually occurs, especially in the case of eggs laid by beetles emerging from the original host, in which case the mortality is often total. One-half to full-grown larvæ, however, usually can be successfully transferred to a new host and live and transform to adults.

(4) With some species that can be reared in a secondary (new) host, by the larvæ feeding one or part of one year, preference for that host is shown by the resulting adults.

(5) In general, the fewer the hosts in nature, the more marked the predilection for a particular host, and vice versa.

(6) Continued breeding in a given host intensifies the preference for that host.

(7) The condition of the host has a great influence on host selection, in that every species prefers an optimum condition of the host which it selects and will choose a new host in the optimum condition in preference to an old host in which the conditions are unfavorable.

(8) The quantity of wood at the disposal of the ovipositing adults may influence the insects in their choice between different kinds of host wood, in that, if there are many adults to a limited amount of the primary host, some species will select a secondary host if such is available. If this is done, however, the resulting brood is weakened.

It is altogether possible that these experiments may indicate the origin of certain closely related species or varieties of insects. For instance, a species restricted to a very few plants may accidentally be forced to

take a new host (as actually happened in the experiments with Cyllene in oak). A few individuals may survive and continue the strain so that it becomes, after a time, at least physiologically different and may also develop correlated differences of color or structure. It can hardly be said that such forms are much less distinct than in the case of the two species *Callidium antennatum* in pine and *C. janthinum* in juniper; for even though these have a slight color distinction and each is absolutely restricted to its own host, they interbreed. On the other hand, in the different forms of *Hylotrupes ligneus*, of which the eastern form in juniper is constant in marking, the western form in redwood is quite variable, as is also the Rocky Mountain form in Douglas fir. The juniper and redwood forms interbreed, but all attempts to mate either of these with the Douglas fir form have failed. All these forms can be furnished with substitute hosts, but in the experiments in which this has been done the original color pattern has resulted thus far.

The grape and hickory strains of *Cyllene pictus*, although showing no color differences, do not readily mate. Two species of Cyllene, *C. pictus* and *C. robiniae*, are separable only as adults, by a slight difference in the color pattern, yet in seasonal and biological habits they are strikingly different. It is conceivable that one of the two species originated through the adoption of a new plant and continuous breeding in that plant.

It may be asked, If one or two years' feeding in a new host results in individuals which prefer that host, thus giving rise at least to new physiological varieties, why does not this occur more frequently in nature? That it does occur must be granted, as we have species living in many host plants as well as those restricted to a species or genus, but that it is not of more common occurrence is believed to be due to the high mortality in first-stage larvæ in a new host rather than to absence of oviposition in the new host. Although the adults show a decided predilection for a favored host in ovipositing and even, in certain species, a preference for the plants in which the larvæ have fed for one or two generations, the instinct to oviposit seems to overbalance that of host selection, consequently new hosts are frequently selected—possibly more frequently in nature than is generally realized. As an example of this, take *Cyllene pictus* requiring hickory cut during the winter. This condition would be fully met in tops left during logging operations. When the timber cutting ceased, a concentration of adults would be left with none of the favored host plant available in the right condition. The grape, osage orange, and hackberry strains collected at Hummelstown, Pa., were in reality taken in a woods which had been logged for hickory and in which operations had ceased three years prior to the finding of these strains. At Falls Church, Va., in June, 1920, adults of *Neoclytus erythrocephalus* were observed ovipositing on pine logs. Much infested ash, from the previous year, was lying about from which they had emerged in great numbers.

NOTES ON THE ORGANIC ACIDS OF PYRUS CORONARIA, RHUS GLABRA, AND ACER SACCHARUM

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During the study of other compounds found in the plants in question, we have incidentally isolated and identified the organic acids of the fruits of the wild American crab apple (*Pyrus coronaria* L.) and the smooth sumac (*Rhus glabra* L.). We have also made an examination of the product known as "maple sand" (found to be impure calcium malate) which is formed as a granular deposit in the pans during the process of boiling down sap of the sugar maple (*Acer saccharum* Marsh.) to make maple sirup. Every precise record of the distribution of plant products is distinctly worth while, and rather than hold our data on the acids of these three plants for incidental mention in papers dealing with other matters, we have thrown them together in the following notes.

OCCURRENCE OF MALIC ACID IN PYRUS CORONARIA, AND ITS TRANSFORMATION INTO SUCCINIC ACID

As might have been predicted from the botanical relationship of *Pyrus coronaria* to the common apple, the very sour fruit of the American crab apple was found to contain malic acid. It was also found that in water extracts of this fruit, made without heat, there is a transformation of malic into succinic acid, apparently through the action of enzyms of the fruit itself. This discovery will be of no little interest if further investigations substantiate our belief that microorganisms were not concerned in the process.

Cold water extractions of crab apples collected near Ann Arbor, Mich., were made in the presence of both chloroform and toluol, with the expectation of obtaining solutions of the fruit acids free from pectin and other colloidal substances. The extractions were made in large stone jars, tightly packed with sliced fruits and filled to the top with water saturated with chloroform and toluol. At the bottom there was an excess of chloroform and at the top an excess of toluol. The solution quickly became intensely sour. The extraction was allowed to take place for several weeks, at the end of which time the apple tissues were as green and hard as when collected, showing no change whatever in appearance. The infusion was brown at the surface layer, but clear and of a pale straw color below. Only the non-oxidized lower part of the

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solution was drawn off through a tubulature at the bottom of the jar. It was not until succinic acid instead of one of the usual fruit acids was isolated from this solution that suspicion arose as to the possibility of any fermentation, other than an autolytic one, having occurred, and it was then too late to examine the solutions for microorganisms. It can only be stated that there was no evidence that such were present. The high acidity would have prevented bacterial action, and the perfectly clear solution, saturated with chloroform and toluol, showed no evidence of the presence of yeasts.

The infusion was neutralized by stirring with calcium carbonate. During this process rapid darkening took place, with the formation, presumably, of the same brown oxidation product that forms when a cut surface of apple is exposed to the air. A dark precipitate separated, which was not a salt of one of the fruit acids. It was filtered off. From the filtrate it was possible to get relatively pure succinic acid by acidifying with hydrochloric acid and shaking with ether, but the more economical and easy procedure, by which a larger yield was obtained, is described below.

The neutralized filtrate was evaporated to a small volume and precipitated with several volumes of ethyl alcohol. An impure calcium salt separated as a sticky, molasses-like mass. It was dissolved in dilute hydrochloric acid, and the solution was concentrated until the succinic acid crystallized out. It was purified by crystallization from 10 per cent nitric acid, and finally by repeated recrystallization from water.

The pure acid thus obtained agreed in all properties with succinic acid. It melted at 184° to 185° C. (Rosenthaler (*13*) [1] gives 185° C.). The reaction mixture obtained by heating with concentrated sulphuric acid, when diluted, boiled, and neutralized with ammonia, gave a red solution with a strong green fluorescence, a characteristic reaction of succinic acid.

Combustions of the pure material dried at 110° C., resulted as follows:

(I) Weight of sample, 0.2378 gm.; H_2O, 0.1047 gm.; CO_2, 0.3556 gm.
(II) Weight of sample, 0.3063 gm.; H_2O, 0.1356 gm.; CO_2, 0.4530 gm.
Calculated for $C_4H_6O_4$; C, 40.66 per cent; H, 5.12 per cent.
Found: (I) C, 40.78 per cent; H, 4.94 per cent. (II) C, 40.33 per cent; H, 4.96 per cent.

Titration with sodium hydroxid (NaOH) gave the following results:

(I) 0.1776 gm. acid required 30.037 cc. *N/10* NaOH.
(II) 0.1453 gm. acid required 24.424 cc. *N/10* NaOH.
Calculated for $C_4H_6O_4$; replaceable H, 1.707 per cent.
Found: (I) 1.703 per cent; (II) 1.692 per cent.

A silver salt was prepared and analyzed, giving the following data:

(I) 0.4809 gm. salt gave 0.3119 gm. Ag.
(II) 0.4818 gm. salt gave 0.3126 gm. Ag.
Calculated for $C_4H_4O_4Ag_2$; Ag 65.02 per cent.
Found: (I) 64.86 per cent; (II) 64.88 per cent.

[1] Reference is made by number (italic) to "Literature cited," p. 228.

It will be observed that if malic acid had been present in the cold water extract, it would have been discarded with the mother liquor from the first crop of succinic acid crystals, since malic acid is not only very deliquescent but likewise difficult to crystallize from solutions containing sugars and other impurities. Some malic acid was doubtless lost at this point, but the large yield of succinic acid indicated that it was the chief acid derived from the fruits which had undergone autolysis.

It was of course a matter of interest to find out whether or not succinic acid was present also in the living fruit. A new supply of crab apples was therefore heated with water in an autoclave at 20 pounds pressure, and the juice, after filtration through cloth, was evaporated to a small volume and treated with several volumes of alcohol, to throw out pectin and other colloids insoluble in alcohol. The alcohol was distilled from the filtrate, which was concentrated, in vacuo, to a sirup. From this sirup it was impossible to obtain even a trace of succinic acid, by either of the methods which had been successfully used with the cold water extracts. It contained, on the other hand, a large quantity of malic acid, identified by the preparation and analysis of its silver salt. The concentrated sirup mentioned above was diluted with water, which brought about a separation of a small precipitate of red pigment, which was filtered off. When lead acetate was added to the filtrate, the acidity of the solution was so great that the first increment caused no precipitation of lead malate but did throw out a small amount of dark precipitate, which was of course removed. Further addition of lead acetate gave a voluminous precipitate of lead malate (A), which was filtered off and washed. A second yield of lead malate (B) was obtained from the solution by the addition of alcohol. The two precipitates were separately decomposed with hydrogen sulphid, neutralized with sodium hydroxid, and silver nitrate solution was cautiously added. The first few drops of the silver nitrate produced a dark precipitate which was removed by filtration. Further addition of silver nitrate caused white silver malate to separate. The precipitates were dried at 105 C. and analyzed as follows (two samples each from A and B):

(I) 0.5317 gm. salt from A gave 0.3315 gm. Ag.
(II) 0.7249 gm. salt from A gave 0.4518 gm. Ag.
(III) 0.3067 gm. salt from B gave 0.1882 gm. Ag.
(IV) 0.5374 gm. salt from B gave 0.3303 gm. Ag.

Found: (I) 62.34 per cent; (II) 62.32 per cent; (III) 61.36 per cent; (IV) 61.46 per cent.

Pure silver malate would have given 62.00 per cent silver. In view of the fact that the acid itself was not purified before the silver salt was formed, the analytical results are sufficiently close. Doubtless other acids than malic are present in very small quantity in the crab apple. The significant fact is that the fresh fruit contains malic acid as the predominant acid, and not enough succinic acid so that we were able to isolate it.

Although the old observations and experiments of Dessaignes (7) and of Liebig (*11*) showed that succinic acid was formed from malic acid when calcium malate was present in mixtures being fermented by yeast, nevertheless succinic acid is generally found in plants in such small quantities and always so intimately associated with asparagin that it is now customary to look upon it as a degradation product of protein rather than as directly related in metabolism to the other plant acids. The possibility of amino compounds giving rise by enzym action in the plant to succinic acid is sufficiently indicated by such discoveries as that of Ehrlich (*8*), who has traced the production of succinic acid by yeast to the fermentation of glutamic acid, and of Harden (*10*), who has shown that putrefactive bacteria (*Bacillus coli communis*), in the presence of glucose, will transform aspartic acid almost quantitatively into succinic acid. However, it must be stated that the whole subject of the place of succinic acid in metabolism is much in need of investigation. It would be a decided step forward to show that it is possible for malic acid to be transformed directly into succinic acid by enzym action, as appears to have taken place by autolysis in the crab apple.

We wish to indicate the possibility that such a transformation takes place and to point out that green fruits containing malic acid afford ideal material for a study of the problem. We do not wish, however, to give the impression that the possibility of fermentation by microorganisms was absolutely excluded in our work.

To anyone who may be inclined to take up the problem of acid transformations in green fruits, a word of caution may not be amiss with regard to the statements that have crept into general reference books such as those of Czapek (*6, p. 434*) and Wehmer (*14*) with regard to the distribution of succinic acid in plants. It is recorded from a number of unripe fruits but has actually been isolated or satisfactorily identified in very few cases. In 1876 Brunner and Brandenburg (*2*) isolated it from the juice of unripe grapes (*Vitis vinifera* L.). The source of most subsequent reports is a paper published in 1886 by Brunner and Chuard (*3*). These authors called attention to the earlier observation of Buignet (*4*) that the juice of green fruits is capable of absorbing a large amount of iodin, which enters into chemical combination with some constituent of the juice. At the same time, a precipitate is formed, which Buignet erroneously supposed to be the iodin compound. Brunner and Chuard, taking up the problem at this point, showed that the iodin compound remained in solution; and they obtained evidence which satisfied them that it was a glucosid of monoiodosuccinic acid, derived from a naturally occurring glucosid of succinic acid. Their investigation covered a considerable number of green fruits and plant juices; and they actually isolated succinic acid, as such, from unripe gooseberries and from the petioles of rhubarb. In the other instances it was merely inferred from analogy that the supposed succinic acid glucosid was present. The procedure

was to treat the juice with lead acetate, which supposedly threw out all iodin-absorbing compounds except the succinic acid glucosid. Then the presence of the latter, which was never isolated at all, was inferred from two circumstances: (1) that the purified juice absorbed iodin, and (2) that, after the absorption of iodin, a precipitate could be obtained with basic lead acetate, supposed to be lead monoiodosuccinate, which when treated with a mineral acid to liberate the free monoiodosuccinic acid, and then with finely divided metallic silver, gave malic acid. The production, under these circumstances, of malic instead of tartaric acid was thought to indicate that iodosuccinic acid had been present rather than an iodin derivative of the widely distributed malic acid.

The weakness of the whole argument is sufficiently obvious without going into detail, since neither the putative glucosid of succinic acid nor the iodosuccinic acid was isolated; and it was not shown that the basic lead acetate precipitate was free from lead malate, which one would naturally expect to be found there. To the physiologist who is interested in the ripening of fruits it will be clear that the whole problem of the distribution and significance in metabolism of succinic acid is much in need of more study. Especially, there can be no doubt that Buignet's iodin-absorbing compound (*4*), whatever it may be, should be taken account of in studies of fruit ripening. It exists in large amount in the unripe fruit and disappears as ripening proceeds. As far as we are aware, it is not even referred to in the recent literature of the subject.

ACIDS OF RHUS GLABRA

The acid of the sour, red pericarp of the sumacs (several species related to *Rhus glabra*) has been variously reported by different investigators as citric, malic, and tartaric. Gallic acid has likewise been reported. The closely related species of true sumacs are doubtless alike as to acid content. Our work, confined to *R. glabra*, has verified the findings of Rogers (*12*) nearly a century ago, and Frankforter and Martin (*9*) that the fruit acid is malic, nearly all in the form of the acid calcium salt. We were also able to isolate free gallic acid, which seems not to have been reported from this particular species. There are statements in the older literature that free gallic acid occurs in the leaves of the European sumac, *R. coriaria* L.

The berries of *Rhus glabra* were boiled with successive quantities of distilled water. The water solutions were clarified and largely freed from tannin by boiling with hide powder and egg albumen, and were then shaken with ether. The combined ether extracts were evaporated to a sirupy consistence and deposited gallic acid as a yellow powder. The latter was filtered off on a Buchner funnel and crystallized repeatedly from water. It was obtained in pure and almost colorless condition by precipitation from solution in absolute alcohol by chloroform, or by recrystallization from glacial acetic acid. As obtained by crystallization

from water it formed brown aggregates of large crystals containing one molecule of water. (Calculated for $C_7H_6O_5 \cdot H_2O$, H_2O, 9.57 per cent; found, 9.39 per cent.) It was identified by the usual tests. Mr. N. A. Lange made combustions of some of the purified acid and of its triacetyl derivative, the results of which he kindly permits us to publish as follows:

I. The acid gave C, 50.19 per cent; H, 3.92 per cent. Calculated for gallic acid: C, 49.40 per cent, H, 3.56 per cent.

II. The acetyl derivative gave C, 53.91 per cent; H, 4.13 per cent. Calculated for triacetyl gallic acid, C, 54.39 per cent; H, 4.06 per cent.

The melting point of the triacetyl gallic acid, stated variously in the literature from 151° to 165° and 166° C., was 162° to 163° C.

After the removal of tannin and gallic acid the aqueous extract from the berries was largely neutralized with calcium carbonate and filtered hot, after considerable concentration. Alcohol threw out a voluminous precipitate, the first fractions taffy-like, later ones solid. These fractions were treated with enough hydrochloric acid to form the acid calcium salt, and were repeatedly treated with animal charcoal and recrystallized from hot water.

The pure crystals were dissolved in water, exactly neutralized with standard alkali; and normal silver malate was precipitated by the addition of silver nitrate. The four successive fractions of the crude calcium salt were designated A, B, C, and D, and each was purified and converted into the silver salt. In addition, a portion of fraction A was purified by further recrystallization and was obtained in two portions called Aa and Ab, from which silver salts were also prepared. The duplicate analytical figures for all of the silver precipitates are given in Table I.

TABLE I.—*Duplicate analyses of silver salts prepared from a series of precipitates obtained by fractional separation with alcohol from an aqueous solution of calcium salts of the organic acid of the sumac fruit*

Fraction.	Weight of silver salt.	Weight of silver.	Percentage of silver.
A–I	0.2895	0.1794	61.96
A–II	.2133	.1318	61.79
Aa–I	.4385	.2721	62.05
Aa–II	1.1358	.7048	62.05
Ab–I	.6137	.3794	61.82
Ab–II	.9674	.5962	61.63
B–I	.4541	.2806	61.79
B–II	.6359	.3932	61.83
C–I	.4242	.2624	61.85
C–II	.5015	.3099	61.79
C–III	.3921	.2420	61.71
D–I	.4195	.2596	61.88
D–II	.5263	.3259	61.92

The figures from all the fractions are in excellent agreement with each other and agree fairly well with malic acid. The results prove beyond much doubt that only one acid is present in any quantity. Rogers (*12*), the first to show the presence of calcium malate in berries of *Rhus glabra*, did not attempt to prove that malic acid was the only one present, and subsequent work was less careful than his. Although convinced by the identity of the silver salts that nothing but malic acid was present in our material, we felt that the determinations should be closer to the calculated value. The average of 13 determinations makes the percentage of silver in the silver salt 61.85, whereas the theoretical value is 62.00 for pure malic acid. We, therefore, prepared silver malate, using a Kahlbaum preparation of the acid, and made four silver determinations in the same manner in which our other determinations were made. The four determinations gave us 61.94 per cent, 61.92 per cent, 61.81 per cent, and 61.91 per cent, averaging 61.89 per cent silver in pure silver malate by our method of preparation and analysis. There can, therefore, remain no doubt that the acid of sumac berries is all malic.

MALIC ACID IN SUGAR-MAPLE SAP

It is no new observation that malic acid is present in the sap of the sugar maple. Cowles (*5*), for example, has published methods for the estimation of malic acid in maple products. Although it might have been anticipated that the granular precipitate known as "maple sand" which is deposited in the pans during the concentration of the sap would prove to be calcium malate, no one, as far as we know, has previously reported an analysis. Bloor (*1*) used "sugar sand" as a source of acid in his work on the transformation of malic acid into sugar by the tissue of the maple[1] but gave no data to bear out the natural and perhaps quite justifiable inference that the acid was actually malic. Our sample was kindly obtained for us from Ohio, by Dr. Clinton A. Ludwig, now of Clemson College, S. C. It was only necessary to add to the "maple sand" sufficient hydrochloric acid to transform the crude calcium malate into the acid calcium salt. The latter was obtained pure by repeated boiling with animal charcoal and recrystallization from hot water. It was neutralized with alkali, and silver nitrate was added to precipitate the insoluble silver malate. Three separate analyses for silver gave the following results:

(I) 0.4129 gm. silver salt gave 0.2560 gm. Ag.
(II) 0.1922 gm. silver salt gave 0.1190 gm. Ag.
(III) 0.2892 gm. silver salt gave 0.1796 gm. Ag.
Calculated for $C_4H_4O_5Ag_2$; Ag, 62.00 per cent.
Found: (I) 62.00 per cent; (II) 61.91 per cent; (III) 62.10 per cent.

[1] It may be noted that Bloor used tissues of "Acer saccarinum" for his work. Since he gives no authority for the name, one is left in doubt as to whether he means the silver maple (*A. saccharinum* L.) or the sugar maple (*A. saccharum* Marsh.; *A. saccharinum* Wang., not L.).

SUMMARY

(1) The acid of the sour fruit of the wild American crab apple, *Pyrus coronaria*, is malic acid. When the fruit undergoes autolysis under anaerobic conditions, in the presence of chloroform and toluol, this acid appears to be transformed largely into succinic acid. Further experiments, however, will have to be made in order to repeat the observations and to determine the exact process involved.

(2) The acid of the outer part of the red fruit of the smooth sumac, *Rhus glabra*, is malic acid, occurring in the form of the acid calcium salt. With it is associated a considerable quantity of free gallic acid.

(3) Malic acid is present in the form of calcium salts (both acid and normal) in maple sap. The product known as "maple sand" obtained from the evaporating pans is crude calcium malate.

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FERTILITY IN SHROPSHIRE SHEEP[1]

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Heape (*4*)[2] mentions that in some breeds young ewes bear fewer twins than older ewes. Carlyle and McConnell (*2*) reported some observations which they had made on the effect of age on fertility in sheep from which they concluded that ewes from 3 to 6 years old averaged a larger percentage of lambs than younger or older ewes, and also that 1-year-old rams were not so prolific as those 2 or 3 years old. The same conclusions were reached by Humphrey and Kleinheinz (*6*) from a study of later records of the Wisconsin flock. Recently Jones and Rouse (*7*) showed that in sheep the percentage of twins increased with age until 5 years, when there was a decided drop.

The present paper gives the results of a study of the influence of age and season upon fertility in American Shropshire sheep.

The source of data is the American Shropshire Sheep Record (*1*). Individuals with registry numbers between 325502 and 344869 have been used, date of birth noted, whether born as single, twin, or triplet, and age of dams and sires looked up.

AGE OF EWE AND FERTILITY

Table I shows the percentage of lambs born as singles, twins, and triplets from dams of various ages. Ewes under 1 year and 6 months are grouped in the 1-year class, those 1 year and 7 months to 2 years and 6 months in the 2-year class, and so on. The percentage in multiple births increases to 4 years and remains fairly constant through 8 years. For the older groups the numbers are too small to draw conclusions.

[1] Paper No. 16 from the Laboratory of Genetics, Agricultural Experiment Station, Urbana, Ill.

[2] Reference is made by number (italic) to "Literature cited," p. 234.

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TABLE I.—*Age of ewe and fertility*

Age of dam in years.	Total number of offspring.	Percentage of singles.	Percentage of twins.	Percentage of triplets.	Percentage in multiple births.
1	379	77.0	23.0	.....	23.0
2	2,299	66.4	33.2	0.4	33.6
3	2,025	63.6	36.1	.3	36.4
4	1,762	57.6	41.4	1.0	42.4
5	1,256	58.0	43.0	1.0	44.0
6	942	53.7	46.0	.3	46.3
7	506	56.3	43.1	.6	43.7
8	405	54.8	44.5	.7	45.2
9	157	62.4	37.0	.6	37.6
10	96	38.5	61.5	.....	61.5
11	23	60.9	39.1	.....	39.1
12	3	100.0	.....	.....	.....
13	4	50.0	50.0	.....	50.0
14	1	100.0	.....	.....	.....
15	4	75.0	25.0	.....	25.0
16	5	20.0	80.0	.....	80.0
20[a]	1	100.0	.....	.....	.....
	9,868	60.8	38.7	.6	39.2

[a] This may be a mistake in the record.

AGE OF RAM AND FERTILITY

Table II gives the percentages of lambs born as singles, twins, and triplets born from sires of various ages. From these percentages one can not ascribe to the ram any influence on fertility. Carlyle and McConnell (*2*) thought that 1-year-old rams were not so prolific as older rams, but this is not borne out by the figures in Table II.

TABLE II.—*Age of ram and fertility*

Age of ram in years.	Total number of offspring.	Percentage of singles.	Percentage of twins.	Percentage of triplets.	Percentage in multiple births.
1	1,101	58.7	40.4	0.9	41.3
2	3,265	60.6	39.0	.5	39.4
3	2,552	59.1	39.9	1.0	40.9
4	1,460	65.8	33.8	.2	34.0
5	650	55.5	43.8	.6	44.5
6	434	66.1	33.6	.2	33.9
7	244	70.5	29.5	.....	29.5
8	118	74.6	25.4	.....	25.4
9	71	63.4	36.6	.....	36.6
10	47	68.1	25.5	6.4	31.9
11	2	100.0	.....	.....	.....
12	3	100.0	.....	.....	.....
	9,947	61.2	38.2	.6	38.8

TIME OF BIRTH AND TWINNING

Heape (*5*), who gathered information from flock masters, states that 55 per cent of them reported that twins were usually born early in the

lambing season. To test this point Table III was made, showing the month of birth and the percentages of singles, twins, and triplets. It is readily seen that a larger percentage of twins is born early in the season than is born later. Of the 3,790 lambs born in January, February, and March 42.3 per cent are twins, while of the 4,617 born in April, May, and June only 36.1 per cent are twins. If the triplets are added in with the twins the percentages are 43.1 in multiple births for January, February, and March, and 36.7 for April, May, and June. As Heape (*5*) points out, this may be due to the ewes with the most vigorous and active generative systems coming into heat earlier in the season. This may be also affected by the fact that early in the breeding season more green feed is available, a factor influencing the number of twins produced.

TABLE III.—*Months of birth (Shropshires)*

Month.	Total number.	Percentage of singles.	Percentage of twins.	Percentage of triplets.
January	33	75.8	24.2	.....
February	471	56.7	43.1	0.2
March	3,286	56.7	42.4	.9
April	3,615	62.4	37.0	.6
May	966	66.3	32.9	.8
June	36	75.0	25.0	.....
August	2	100.0	.....	.....
September	1	100.0	.....	.....
December	7	100.0	.....	.....

In the hope that additional information might be obtained, a study was made of the Dorset breed (*3*), which produces a large number of young in the fall. Table IV gives the month of lambing and the percentages of singles, twins, triplets, quadruplets, and of all multiple births.

TABLE IV.—*Months of birth (Dorsets)*

Month of birth.	Total number.	Percentage of singles.	Percentage of twins.	Percentage of triplets.	Percentage of quadruplets.	Percentage in multiple births.
January	1,818	61.5	37.1	1.3	0.1	38.5
February	2,386	54.3	41.9	3.8	.....	45.7
March	3,919	52.7	43.9	3.2	.2	47.3
April	2,366	51.7	45.4	2.6	.3	48.3
May	857	54.8	43.1	2.1	.....	45.2
June	296	59.8	38.5	1.7	.....	40.2
July	90	65.6	27.8	6.6	.....	34.4
August	102	68.6	30.4	1.0	.....	31.4
September	925	73.7	25.3	.9	.1	26.3
October	1,546	66.2	32.9	.9	.....	33.8
November	1,088	67.7	30.5	1.8	.....	32.3
December	1,418	61.0	36.8	2.2	.....	39.0
	16,634	57.8	39.7	2.4	.1	42.2

From Table IV it can be clearly seen that the percentage of multiple births is greater in the spring. If the births occurring from February to June, inclusive, are combined it is found that 48.2 per cent are in multiple births, while for the other months the percentage is 34.9. In Shropshires a larger percentage of twins or multiple births occurs in January, February, and March than later. This condition does not seem to hold for the Dorsets. Therefore, this condition in the Shropshires is not likely due to more green feed early in the mating season. The causes of these significant differences in multiple births at different seasons among sheep are yet to be discovered.

SUMMARY

(1) Multiple births increase with age up to 4 years. From this point they remain fairly constant until 8 years. Beyond this age the numbers are too small to draw conclusions.

(2) The age of the ram has no influence on the percentage of multiple births.

(3) Among Shropshire sheep more multiple births occur early in the lambing season than later.

(4) Among Dorsets more multiple births occur in spring than in fall.

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